

Gingipain-dependent interactions with the host are important for survival of *Porphyromonas gingivalis*

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1. ABSTRACT

Porphyromonas gingivalis, a major periodontal pathogen, must acquire nutrients from host derived substrates, overcome oxidative stress and subvert the immune system. These activities can be coordinated via the gingipains which represent the most significant virulence factor produced by this organism. In the context of our contribution to this field, we will review the current understanding of gingipain biogenesis, glycosylation, and regulation, as well as discuss their role in oxidative stress resistance and apoptosis. We can postulate a model, in which gingipains may be part of the mechanism for *P. gingivalis* virulence.

2. INTRODUCTION

P. gingivalis, a black-pigmented, Gram-negative anaerobe, is an important etiological agent of periodontal disease and is also linked to cardiovascular disease and other systemic diseases [reviewed in (43,103)]. The inflammatory nature of periodontal disease implies that innate host defense mechanisms play a vital role in limiting bacterial growth. During active infection including tissue invasion, toxic reactive oxygen metabolites (e.g. superoxides, hydrogen peroxide and hydroxyl radicals) are mostly generated by polymorphonuclear leukocytes and macrophages (27,29). In order to survive in the inflammatory environment of the periodontal pocket, the

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bacterium must not only obtain nutrients for growth, but also overcome oxidative stress and subvert the immune defense system. Coordinated regulation of these activities would be beneficial to the bacterium. While there is documented evidence of the response of *P. gingivalis* to environmental stimulus (56,117,126), one possible mechanism for coordination may occur via the gingipains produced by this bacterium. The presence of *P. gingivalis* in the periodontal pocket and the high levels of gingipain activity detected in gingival crevicular fluid could implicate a role for gingipains in the destruction of the highly vascular periodontal tissue. Protease-associated degradation of cell-cell adhesion proteins on epithelial and endothelial cells resulting in cell death will compromise tissue integrity and facilitate spreading of the bacterium. Furthermore, degradation of the immune response components will facilitate the survival of the organism. Together, these activities may also satisfy the nutritional requirements of the organism. Gingipain-dependent heme accumulation on the bacterium cell surface may also act as an “oxidative sink”, thus, leading to protection against oxidative stress (191,193). We will discuss the role of the gingipains in the survival/pathogenicity of *P. gingivalis* and the unique *vim* (virulence modulating) locus that is involved in regulation of the gingipains. We will highlight some of our observations that are consistent with the hypothesis that the gingipains are central to an effective survival strategy for the organism.

3. *P. GINGIVALIS* VIRULENCE FACTORS - GINGIPAINS

While several virulence factors [(fimbriae (adhesins), capsule (antiphagocytosis), lipopolysaccharide (bone resorption), proteases (specific and generalized tissue destruction) and a variety of toxic by-products (e.g., ammonia)] have been implicated in the pathogenicity of *P. gingivalis*, the high proteolytic abilities of this organism are considered to play the most significant role in virulence [reviewed in (85,142)]. Proteolytic enzymes that have been secreted by this organism include endopeptidases, aminopeptidases, carboxypeptidase, oligopeptidase, and di- and tri-peptidyl peptidases [reviewed in (161)]. The major proteases are endopeptidases called gingipains, (*P. gingivalis* +clostripain) and are both extracellular and cell-associated. They consist of arginine-specific proteases [Arg-gingipain, (Rgp)] and lysine-specific protease [Lys-gingipain, (Kgp)]. The Rgp is encoded by two genes *rgpA* and *rgpB* and Kgp is encoded by a single gene *kgp* (142). The gingipains have multiple functions [reviewed in (50,80,142,161)]. In addition to being essential for growth, because of the asaccharolytic nature of this organism, they play a role in complement and immunoglobulin degradation, inactivation of cytokines and their receptors, platelet aggregation, attenuation of neutrophil antibacterial activities, and increasing vascular permeability, as well as, prevention of blood clotting. We and others have also demonstrated the ability of the gingipains to disrupt cell-cell and cell-matrix adhesion and induce apoptosis in several cell types (34,99,129,182,183). An involvement in hemoglobin binding and adsorption and heme accumulation further confirms their multiple contributions to bacterial

survival, including oxidative stress resistance (3,107,114,152,191).

3.1. Gingipain biogenesis/activation

The translated product of the *rgpA* gene is composed of a pre-pro-fragment, followed by a proteinase domain, and a large hemagglutinin/adhesion (HA) domain at the C-terminus (40,162). The *kgp* gene product shares a similar size and structure to the RgpA gingipain. While it displays a 20% identity in the catalytic domain compared to RgpA, the amino acid sequence of the C-terminal HA domains are identical (157,158). As demonstrated in Kgp, the C-terminal HA domains of RgpA are not only essential for full expression of gingipain activity, but also for proper processing of the multiprotein complex assembly on the *P. gingivalis* outer membrane (199). In comparison to *rgpA*, the *rgpB* gene is missing almost the entire section coding for the HA domain, with the exception of a small C-terminal segment (132,141). Collectively, these observations indicate that the mature gingipains are derived from extensive processing from the nascent polypeptide precursors. Studies comparing the gene structure with the N-terminal sequences of different isoforms of the purified gingipain have demonstrated specific post-translational cleavage at Arg-Xaa and Lys-Xaa bonds (20). There is emerging evidence that this post-translational process is achieved by an autoproteolytic mechanism (131). Further, both gingipains R and K can be mutually involved in the efficient maturation of each other in addition to a carboxypeptidase (CPG70) that can play a role in the C-terminus processing of RgpA and Kgp HA domains (219).

As a common theme, the expression of extracellular proteolytic activities is highly regulated in both prokaryotic and eukaryotic systems [reviewed in (39,221)]. This regulation can occur at multiple levels including processing of an inactive secreted precursor to its active form and/or the post-translational glycosylation of the proteins (60,210). The multiple layers of regulation are vital to ensure that expression is tightly controlled in the appropriate temporal and spatial patterns. Currently, information is known only on the activation pathway of pro-RgpB (131). The recombinant, full length zymogen undergoes three sequential autoproteolytic, intermolecular processing steps that involve processing at Arg¹²⁹, followed by Arg²²⁹, then the C-terminal extensions, to generate full activity. Each step in the processing requires the previous step, and enzyme activity is enhanced in a stepwise manner (131). If gingipain activation occurs by an autoproteolytic mechanism, questions are raised on how this process is regulated and the involvement of specific bacterial host factors. In fact, in previous reports, we have demonstrated the secretion of the inactive proenzyme gingipain species in *vimA*-, *vimE*- and *vimF*-defective mutants (150,213,214,215). In both the *vimE* and *vimF* isogenic mutants, activation of the gingipain proenzyme species could not be achieved (213,214). Taken together, these observations suggest a role for the Vim proteins in the regulation of gingipain activation; however, their mechanisms are unclear and are the subject of ongoing investigation (see further discussion below in section 3.3.).

3.2. Gingipain glycosylation

Glycosylation is one of the important ways by which protein maturation and other cellular processes are regulated (71,201,217). There is emerging evidence that this process may also be important in gingipain biogenesis in *P. gingivalis* (60,213,214,215). For glycosylation, different glycosyl transferases catalyze the transfer of different carbohydrate moieties from active donors to specific acceptors (including lipids, proteins and nucleic acids) (26). In proteins, for example, this can have significant effects on the intrinsic properties that can influence their stability, resistance to proteolysis, and tertiary structure which can affect the rate of processes that involve conformational changes (112,134,200). The level of glycosylation can also modulate the interaction of the glycoconjugate with other molecules (14,189). Given the significant impact of carbohydrate modifications on protein structure/function, glycosylation of bacterial proteins is of major relevance to the molecular basis of pathogenesis. There is a growing list of bacterial virulence factors that are glycoproteins (60,201). Curtis and colleagues were the first to demonstrate that the gingipains are post-translationally modified with carbohydrate additions that are cross-reactive with monoclonal antibodies to *P. gingivalis* lipopolysaccharide (LPS) (41,60). Our recent publications confirm and extend these results (213,214,215). Most of the LPS-like glycan moieties appear to occur at the C-terminus of the polypeptide chain and seem to serve to anchor the gingipain molecule into the outer membrane (146,178,188). There is also diversity and variable levels of carbohydrate modifications demonstrated in the various isoforms of the catalytic domains of Rgp (41,60). This may partly account for the heterogeneity in the RgpA and RgpB isoforms.

There is currently no information on the enzymology of gingipain glycosylation. However, the gene products of *wpbB* and several genes in the *porR* locus (31,60,188) have been shown to participate in modification of the gingipains. These mostly conserved groups of enzymes in Gram-negative bacteria are known to be involved in the biosynthesis of the O-antigen side chains of the LPS component of the outer membrane. Isogenic mutants defective in these genes result in the loss of pigmentation, glycan synthesis and glycosylation, and reduced gingipain activity, which is mostly soluble. In our studies, we have identified other novel genes (*vimA*, *vimE* and *vimF*) that also appear to participate in modification of the gingipains (213,214,215). While the *vimA*-defective mutant displayed a similar phenotype to the *porR* mutant, the gingipains in the *vimE* and *vimF* mutants, although inactive, were cell-associated. Further, there was no observed late onset of gingipain activity in *vimE* and *vimF* mutants suggesting that other modifications beyond those that facilitate membrane anchorage are needed for activation. *VimF* is a putative glycosyl transferase; however, its specific role in glycosylation leading to gingipain activation is unclear. Ongoing studies are testing the hypothesis that regions of the gingipain require specific glycosylation to facilitate activation/maturation. Consistent with this hypothesis is the recent observation that truncation of the last two residues (valyl-lysine) from the C

terminus of RgpB is sufficient to create an inactive version of the protein that lacks the post-translational glycosylation seen in the wild type, and the protein remains trapped behind the outer membrane. Alanine scanning of the last five residues also revealed the importance of the C-terminal motif in mediating correct post-translational modification of the protein (146). RgpB is required for the normal post-translational glycosylation of Arg-gingipains derived from *rgpA* (165). It is unclear how the *vim* genes are involved in this process.

3.3. Regulation of gingipain biogenesis and virulence in *P. gingivalis* - Role of the *bcp-recA-vimA* region

Although several virulence regulators have been described in *P. gingivalis* [reviewed in (56,81)], there is an emerging view that modulation of virulence in *P. gingivalis* may be coordinated via an ability to modulate proteolytic activity. For example, there is evidence that the gingipains can modulate heme uptake/utilization which is vital for its survival [(151) and reviewed in (152)]. Fimbrial expression is also regulated by the gingipains (229). Other studies in our laboratory have also shed light on the relationship of virulence and the ability of *P. gingivalis* to manage the oxidative stress typical of the inflammatory microenvironment of the periodontal pocket. A coordinate relationship between gingipain activity, oxidative stress resistance and virulence is further confirmed by the gene cluster at the *bcp-recA-vimA* locus. We have demonstrated that the *recA* gene plays the expected role in DNA repair (55) (see section 4. below). The *bcp* and *vimA* (virulence modulating) genes are part of the same *recA* transcriptional unit (1) and upstream of the *vimE* and *vimF* genes (213,214). While *P. gingivalis* FLL301, the *bcp*-defective mutant, showed *in vitro* sensitivity to hydrogen peroxide, its virulence potential in a mouse model was unaltered when compared to the wild-type (95). In contrast, the *vimA*- *vimE*- and *vimF*-defective mutants were non-black-pigmented and showed decreased gingipain activity (1,213,214). The gingipain proenzyme species were observed in these mutants providing some of the first evidence for post-translational regulation of protease activity in *P. gingivalis*. It is also consistent with other observations which suggest that glycosylation plays an important role in post-translational regulation of gingipain activity (60,188). In *in vivo* experiments using a mouse model, the *vimA*-defective mutant (*P. gingivalis* FLL92) was dramatically reduced in virulence when compared with wild-type W83 strain (1). We have also demonstrated that *VimA* interacts with the gingipains and other proteins known to be associated with sugar metabolism and protease maturation and is not cleaved by purified RgpB (216). In our bioinformatic studies, *VimA* appears to be a unique protein that lacks DNA binding motifs or known enzyme domains. It may be a putative membrane protein and the C-terminus may also have a putative protein binding domain (216). These data suggest that *VimA* may be part of a protein complex that is involved in gingipain biogenesis/glycosylation (Figure 1).

Collectively, our observations indeed support the hypothesis that regulation of virulence in *P. gingivalis* may be coordinated via an ability to modulate proteolytic

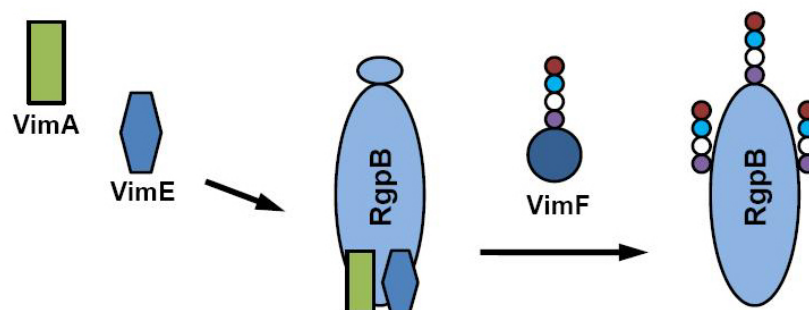


Figure 1. Putative model for VimA-dependent glycosylation of the gingipains. Glycosylation of gingipains are altered by inactivation of the *vimA* gene. It is likely that VimA could function as an accessory protein that will facilitate the interaction of other proteins (e.g. VimF) that is vital for glycosylation/activation. Possible defects of specific glycosyl transferases may prevent the gingipains from folding correctly resulting in lack of activation.

activity, although we cannot rule out any other direct or indirect effect of the *vim* genes on the expression of other virulence factors. It is interesting that the *recA* and *bcp* genes are part of the same transcriptional unit with the *vim* genes that are involved in modulating proteolytic activity. This kind of association may be significant since a response to oxidative stress will involve binding of oxygen and its toxic derivatives to iron accumulated on the surface of the cell via the gingipains (193). The bound heme can be involved in the catalytic destruction of the toxic oxygen derivative species (191). Since proteolytic activity in *P. gingivalis* is associated with heme accumulation (152) via the HA2 hemagglutinin domains of Arg- and Lys- gingipains (192), it might be considered an important strategy for the organism to coordinate its oxidative stress and proteolytic activities. Under conditions of oxidative stress, there is an increased proportion of cell-bound gingipain (RgpA and Kgp) activity (45). This importance is further underscored by the observation that the *recA* gene is expressed during infection of the murine host (118). Further, expression of the *recA* gene is regulated by temperature, iron, and calcium (117) which are components known to coordinately regulate the expression of other bacterial virulence genes [reviewed in (56,126)].

4. THE SIGNIFICANCE OF OXIDATIVE STRESS IN THE SURVIVAL OF *P. GINGIVALIS*

In the inflammatory microenvironment of the mouth, reactive oxygen species (ROS) which are mostly produced by polymorphonuclear leukocytes and macrophages (30), constitute an important component (133). An increase in ROS or depletion of antioxidant molecules and/or enzymes results in oxidative stress. Thus, oxidative stress occurs when there is an imbalance between oxidant exposure and antioxidant protection. In addition, the occasional exposure of *P. gingivalis* to air can give rise to the metabolic conversion of atmospheric oxygen to ROS inside bacterial cells. ROS are highly reactive and can damage proteins, lipids, RNA and DNA (27,133). The generation and accumulation of O_2^* , H_2O_2 and hydroxyl radicals (16,30,133) that target DNA, cellular membranes, metalloproteases and transcription factors (69,86,87,123,195) can be lethal to the organism. In

bacteria, DNA damage appears to be the most significant consequence of oxidative stress [reviewed in (133)]. While oxidant-induced DNA damage generates over 20 different oxidatively altered bases (44), 8-oxoG is the major product of DNA oxidation (179). Unlike some other modified DNA bases, 8-oxoG does not block replication. Instead, it can Watson-Crick base pair with cytosine, as well as, Hoogsteen base pair with adenine (68,205). The polymerases can efficiently incorporate both cytosine and adenine across from 8-oxoG. Mispairing with adenine often leads to GC \rightarrow TA transversion mutations that can be dangerous for the cell (68). Because the average G + C content of the genome of *P. gingivalis* is 49% [(145), (www.oralgen.lanl.gov)], a mechanism(s) to prevent or repair lesions resulting from guanine oxidation is imperative. This is further underscored by the observations that the salivary levels of 8-oxoG; *P. gingivalis* and *T. forsythia* in the periodontitis patients were significantly higher than those in healthy subjects (173). In addition, 8-oxoG was significantly correlated with the presence of *P. gingivalis* (173). Thus, it is crucial that *P. gingivalis* utilize an arsenal of mechanisms to either prevent or fix oxidative damage resulting from ROS in order to survive in this hostile environment. *P. gingivalis* has developed various strategies some of which involve the gingipains in protecting itself from oxidative stress. These strategies include antioxidant enzymes, the hemin layer, DNA repair enzymes, the *bcp-recA-vimA* locus, *htrA*, *feoB2*, and other hypothetical genes of unknown function.

4.1. Oxidative stress resistance: antioxidant enzymes and hemin accumulation

In the first report to describe oxidative DNA damage in an anaerobe, *Prevotella melaninogenica* is shown to be highly sensitive to O_2 or H_2O_2 exposure (203). Under these conditions, the elevated 8-oxoG detected in that strain correlated with its decreased survival. This suggests that oxidative DNA damage is an important cause of oxygen intolerance in *P. melaninogenica*. In our studies, we have also identified the presence of 8-oxoG in the chromosomal DNA of *P. gingivalis* W83 and the non-pigmented isogenic mutant FLL92 after exposure to H_2O_2 (97).

Two cellular systems function in bacteria to protect against oxidative stress (12,119,171). In one system, antioxidant enzymes such as superoxide dismutase (SOD), catalase, peroxidase, and oxidase attempt to neutralize molecular oxygen and ROS (2,4,51,177) before they can damage cellular components. The other system, which will be discussed later, involves endonucleases which are involved in the repair of nucleic acids that have already been damaged (21,105,119), as seen in *Escherichia coli*. Although *P. gingivalis* is oxygen tolerant and is missing catalase activity, it has been shown to express cytosolic SOD activity (35). This SOD activity, however, is protective only for atmospheric oxygen but ineffective against H₂O₂ or exogenously generated ROS (121,143). Recently, rubrerythrin (a non-heme iron protein which functions as a cytoplasmic peroxidase), encoded by the *rbr* gene, and DNA binding proteins, encoded by the *dps* gene, were shown to provide oxidative stress protection against H₂O₂ (198,209). Our own studies (96) have investigated the ability of Ahp (alkyl hydroperoxide reductase), a peroxide-scavenging enzyme, to protect against oxidative damage. This enzyme consists of two components, AhpC with peroxidase activity and the flavoprotein AhpF. Our findings suggest that the *ahpC* gene may play an important role in peroxide resistance in *P. gingivalis* *in vitro*, but does not contribute to its virulence *in vivo*. These results were partly confirmed recently by Diaz, *et al* (47) who demonstrated that *P. gingivalis* seems to lack a protective NADH oxidase but AhpF-C could contribute to its moderate tolerance to reactive oxygen species by metabolizing H₂O₂.

Protection against oxidative damage may also utilize another unique mechanism in *P. gingivalis*. Cell surface heme acquisition has been postulated to be a defense mechanism against ROS in *P. gingivalis* (191,193). The storage of the heme on the cell surface which gives the organism its characteristic black pigmentation, can form μ -oxo dimers in the presence of ROS and can give rise to the catalytic degradation of H₂O₂ (191). An important component of the heme acquisition system in *P. gingivalis* involves the gingipains (65,115,148). Recent reports suggest that gingipains Kgp and RgpA are the major proteases involved in hemin acquisition, binding and accumulation in *P. gingivalis* (24,42,115,163,186,192). This raises an important question as to whether this organism would be more susceptible to oxidative stress in the absence or reduction of this "oxidative sink" layer. Because the gingipains are downregulated at elevated temperature, typical of the inflammatory microenvironment of the periodontal pocket (104,159), or when *P. gingivalis* contacts host cells (156), this may have implications for its capacity to adapt to conditions of oxidative stress. In our studies, we sought to determine if this organism was more susceptible to oxidative stress in the absence or reduction of this hemin layer. Thus, we examined a non-pigmented *vimA*-defective isogenic mutant of *P. gingivalis* (FLL92) which has reduced cell surface associated gingipain activity. As expected, the 8-oxoG and its repair activity were elevated in the non-pigmented isogenic mutant (97), which could further support a protective role of the pigmented layer and, consequently, gingipain involvement in oxidative stress resistance.

4.2. DNA repair mechanisms in oxidative stress

Although vital for survival, there is currently a gap in our understanding of mechanism(s) used by *P. gingivalis* or other anaerobes to repair oxidative DNA damage. It is unclear if the gingipains are coordinately regulated with this process given its association with the formation of the "oxidative sink" layer (191,193). In other bacteria DNA damage can be repaired by several mechanisms including base excision repair (BER) and nucleotide excision repair (NER) (21,106,139,174).

In general, most oxidative DNA damage, whether it is base or sugar damage or the formation of abasic sites, is repaired by BER (106). DNA lesions generated as a result of ROS damage are usually not bulky DNA lesions and are excellent substrates for the process of BER (69). BER involves MutM, MutY, and MutT. MutM removes 8-oxoG paired with cytosine. If replication occurs without the removal of 8-oxoG, MutY, a glycosylase, removes adenine mispaired with 8-oxoG. Thus, MutM and MutY work together to prevent GC \rightarrow TA transversions associated with 8-oxoG. MutT hydrolyzes 8-oxodGTP to 8-oxodGMP, thus depleting the nucleotide pool of 8-oxodGTP. DNA N-glycosylases are the effectors of BER and are more specific in lesion recognition and repair than the process of NER (106).

NER is a universal DNA repair mechanism found in all kingdoms of life (15,206). It is different from all the other forms of DNA repair in its ability to act on a wide variety of substrates (15). NER was one of the first repair mechanisms discovered in bacteria (180) and is mediated by the products of the following genes: *uvrABC*, *uvrD*, *polA* and *lig* (15,172). NER recognizes distortions in DNA caused by bulky adducts that also alter the chemistry of the DNA. Damaged DNA is recognized, cleaved 3' and 5' to the DNA lesion, and repaired in a sequential multistep process that requires ATP (15,172).

In a previous report, we have determined the repair of 8-oxoG-induced DNA damage in *P. gingivalis* (97). Bacterial extracts from the *P. gingivalis* isogenic strains grown in the presence or absence of H₂O₂ were used in glycosylase assays with a 5'-end labeled [γ -³²P]-ATP 8-oxodG:C containing oligonucleotide (24mer) (97). If 8-oxoG is removed by a BER mechanism, a cleavage product corresponding to a 12mer would be observed because the 8-oxodG:C was placed in the middle of the 24mer. The Formamidopyrimidine-DNA glycosylase (Fpg) enzyme generated the expected 12mer fragment in oligonucleotide containing 8-oxoG; however, a fragment of a similar size was missing in *P. gingivalis* strains W83 and FLL92. Instead, a cleavage product of approximately 17 bases was observed. In addition, when compared to other anaerobic periodontal pathogens, the removal of 8-oxoG was unique to *P. gingivalis*. Collectively, these data indicate that the repair mechanism of 8-oxoG is different in *P. gingivalis* when compared to *E. coli* and suggests a mechanism that needs to be defined. In contrast to MutY and MutT, there is no detectable evidence of the *E. coli* *mutM* homologue in the *P. gingivalis* genome [(145), (<http://www.oralgen.lanl.gov/>)]. Preliminary studies in the

laboratory have demonstrated that as in *E. coli*, MutY appears to play a similar role in protecting against oxidative stress in *P. gingivalis* (168).

The *P. gingivalis* genome contains genes which encode for the UvrA, UvrB, and UvrC proteins (145) (<http://www.oralgen.lanl.gov/>). UvrB is the central component of bacterial NER. It is directly involved in distinguishing damaged from undamaged DNA and guides the DNA from recognition to repair synthesis [reviewed in (206)]. To further evaluate if NER may play a role in this repair activity given the size of the cleavage product (97), the *uvrB* gene in *P. gingivalis* was inactivated (78). In contrast to the wild-type *P. gingivalis* W83, the *uvrB*-deficient mutant FLL144 was significantly more sensitive to UV irradiation. However, the enzymatic removal of 8-oxoG was unaffected by the inactivation of the *uvrB* gene. Collectively, these results suggest that the *uvrB* gene in *P. gingivalis* may not be involved in the removal of 8-oxoG and that another yet unidentified mechanism may be employed in its repair.

4.3. Other mechanisms: role of the *bcp-recA-vimA* locus, *htrA*, and hypothetical genes of unknown function in oxidative stress resistance

In *P. gingivalis*, oxidative stress was found to modulate the expression of several proteins including HtpG, GroEL, DnaK, AhpC, FeoB2, TPR domain protein, and trigger factor (8,46,77,82,101,149,184). Since several of these proteins are also involved in heat shock, it implies that the organism uses a multifunctional approach that may involve the gingipains to maintain cell viability under adverse environmental conditions.

Since the *recA* gene product is a key protein in DNA repair, we hypothesized that this DNA repair ability may play a role in the survival and virulence of *P. gingivalis*. We constructed a *recA*-deficient mutant by allelic exchange mutagenesis and showed that in contrast to the wild-type strain, this mutant was more sensitive to UV irradiation (55). Thus, this gene may play the expected role in DNA damage repair (55). We have also identified other genes, *bcp* and *vimA-vimE-vimF* that are part of the same *recA* transcriptional unit and can be differentially expressed (1,213,214). The bacterioferritin comigratory protein (Bcp) is a part of the thiol-specific antioxidant (TSA)/alkyl hydroperoxidase family and functions in a similar way to the AhpC in detoxifying H₂O₂ (92,96). Preliminary evidence has shown that *P. gingivalis* FLL301, a *bcp*-defective mutant, showed increased *in vitro* sensitivity to H₂O₂ (95). The *vimA*-defective mutant also showed increased sensitivity to H₂O₂ compared to the wild-type strain; however, the level of resistance is inducible (125).

In protein-protein interaction studies, the VimA protein was shown to interact with gingipains and other proteins including high temperature requirement A (HtrA) (216). In several organisms, including bacteria, yeast, plant, and humans, HtrA is considered an important factor that is involved in protein folding and maturation as well as in the degradation of proteins that are misfolded (160). It functions as a molecular chaperone at low temperatures and

has proteolytic activity at elevated temperatures (102). Inactivation of the *htrA* gene has been shown to affect the sensitivity of many organisms to thermal and oxidative stress (116,155). In *P. gingivalis*, HtrA is also involved in resistance to thermal and oxidative stress (169). *P. gingivalis* FLL203, an *htrA*-defective mutant, had increased sensitivity to H₂O₂ and decreased Rgp activity. HtrA was also shown to physically interact with the gingipains RgpA, RgpB and Kgp (169). This further underscores the importance of the gingipains and their regulator *vim* genes in oxidative stress resistance in *P. gingivalis*. Thus, we can envision a scenario in *P. gingivalis* where the HtrA protein may be important for regulation of gingipain activity in the inflammatory microenvironment of the periodontal pocket. A specific mechanism for this interaction is unclear and should be further investigated.

Transcriptome analysis of *P. gingivalis* in the presence of hydrogen peroxide has further extended our understanding of the complex response of this organism to oxidative stress (8,46,184). Diaz, *et al* (46) in their DNA microarray analysis of *P. gingivalis* isogenic mutants, have demonstrated that common OxyR-regulated genes such as *dps* and *ahpFC* were not positively regulated in response to H₂O₂, although, their expression was dependent on the presence of a functional OxyR protein. As confirmed elsewhere (147), they concluded that OxyR does not act as a sensor of H₂O₂ in *P. gingivalis*. In preliminary experiments, we have shown that the duration of oxidative stress can modulate different sets of genes (125). As expected, an up-regulation of DNA repair/modification genes and several hypothetical genes which have not been previously characterized were mostly seen at a shorter H₂O₂ exposure time. However, during prolonged exposure to H₂O₂, several genes some of which are known to be involved in protein repair and many previously uncharacterized were up-regulated. Characterization of the hypothetical genes should lead to a more comprehensive understanding of mechanisms of oxidative stress resistance in *P. gingivalis*. It is of particular interest to identify transcriptional regulators that activate transcription of oxidative-stress-related genes in response to H₂O₂. This is being further being investigated in the laboratory. The mechanisms that *P. gingivalis* employs to combat the effects of ROS are just beginning to be elucidated. It will be interesting to determine the extent of gingipain involvement in these processes.

5. GINGIPAIN-INDUCED EFFECTS ON HOST CELL SURVIVAL

5.1. Gingipain-induced degradation of cell adhesion molecules

5.1.1. Gingipains directly cleave Cell Adhesion Molecules (CAMs)

CAMs mediate cell-cell interaction, cell-matrix interactions, and cell signals for growth, differentiation, and apoptosis [reviewed in (164)]. There are four major families of CAMs: cadherins, integrins, immunoglobulin

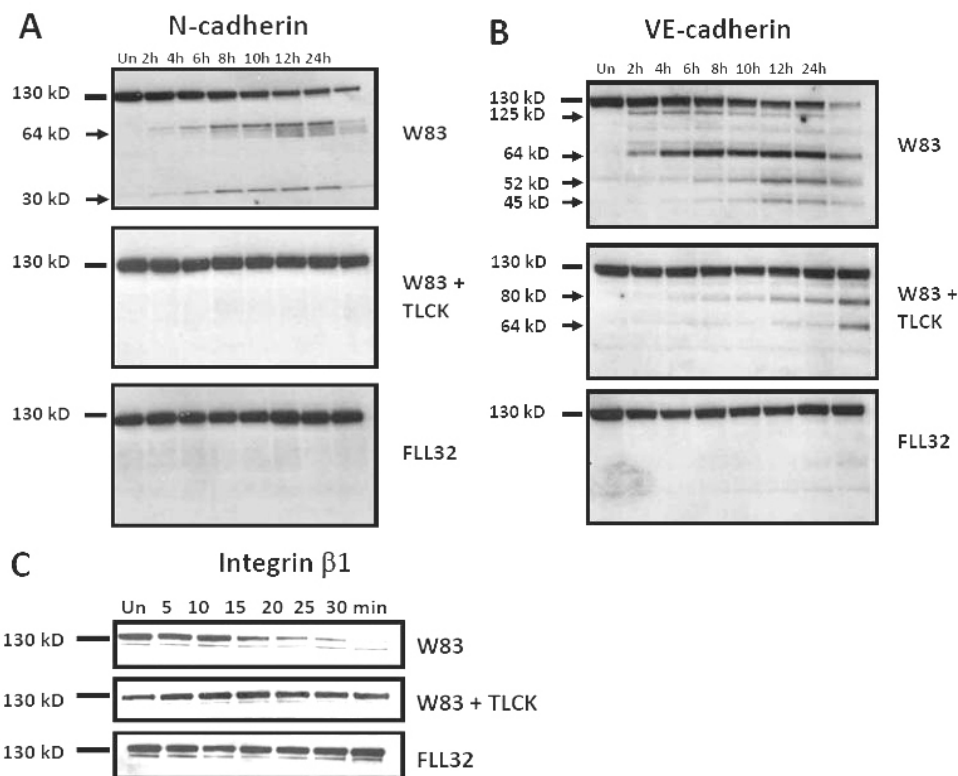


Figure 2. Cleavage of N-cadherin, VE-cadherin, and integrin $\beta 1$ by gingipain-active extracts from strain W83. 10 μ g of protein from BCAEC treated, in the presence of 5 mM L-cysteine, for the indicated times with W83 extracts (70 Units Rgp activity/ml media and 5.3 units Kgp activity/ml media), TLCK-treated W83 and FLL32 extracts (0.73 Units of Rgp activity/ml media and 0.49 Units of Kgp activity/ml media) were separated by SDS-PAGE and immunoblotted with a monoclonal antibody to (A) N-cadherin or (B) a polyclonal antibody to VE-cadherin. (C) BCAEC treated with 200 μ g/ml W83 extract (230 Units of Rgp activity/ml media and 19.4 Units of Kgp activity/ml media), FLL32 extract (0.73 Units of Rgp activity/ml media and 0.49 Units of Kgp activity/ml media), and W83 extract pretreated with 10 mM TLCK were harvested, after treatment for the indicated times, and 20 μ g was separated by SDS-PAGE. Membranes were reacted with a monoclonal antibody to integrin $\beta 1$. Arrows indicate cleavage products. Un, untreated. Reproduced with permission from ASM (182).

gene superfamily members, and selectins. Proteolytic cleavage of these proteins can modulate their signaling functions (130,226). Thus, gingipain-induced degradation of CAMs could be implicated in periodontal disease pathogenesis. There is evidence that degradation of proteins of cell-cell junctional complexes by *P. gingivalis* may facilitate the organism's invasion and spread to deeper structures via a paracellular pathway (98).

We and others have shown that gingipains cleave or modulate the expression of CAMs in various cell types (34,79,83,99,100,182,183,202,220,222). There is specific affinity and cleavage efficiency of the gingipains for CAMs. HRgpA was found to be 12 times more effective than RgpA in cleaving integrin $\alpha 5 \beta 1$ on human gingival fibroblasts, while $\alpha v \beta 3$ was unaffected (176). This study also established that the adhesion domain of HRgpA facilitates binding to integrin $\beta 1$ (176). However, in human gingival fibroblasts Rgp was implicated in the cleavage of integrins $\alpha 2$, $\beta 1$, and $\beta 3$, but not $\alpha 5$ and αv (10). Yun *et al.* (236) showed that in human umbilical vein endothelial cells (HUVEC), RgpA and Kgp degrade CD99, a CAM that has

been implicated in multifactorial cellular events producing prominent contraction and progressive intercellular gap formation in the endothelial monolayer (232). These investigators also demonstrated that endothelial leukocyte adhesion molecule-1 (ELAM-1 also known as E-selectin), VCAM-1, and ICAM-1 were not degraded by the gingipains (232). These effects may contribute to the successful colonization of *P. gingivalis* by limiting the host inflammatory response (232).

We have also demonstrated that treatment with gingipain-active W83 extracts of both coronary artery (BCAEC) and microvascular (HMVEC) endothelial cells resulted in detachment and BCAEC displayed cleaved N-cadherin, VE-cadherin, and degraded integrin $\beta 1$ (182,183). BCAEC integrin $\beta 1$ was cleaved rapidly after exposure to gingipain-active W83 extracts with complete degradation by approximately 30 min (Figure 2C). This degradation occurred when BCAEC were still fully adherent. VE-cadherin cleavage was evident at 2 h and progressed to almost total disappearance of the full length protein by 24 h (Figure 2B). N-cadherin cleavage occurred somewhat later

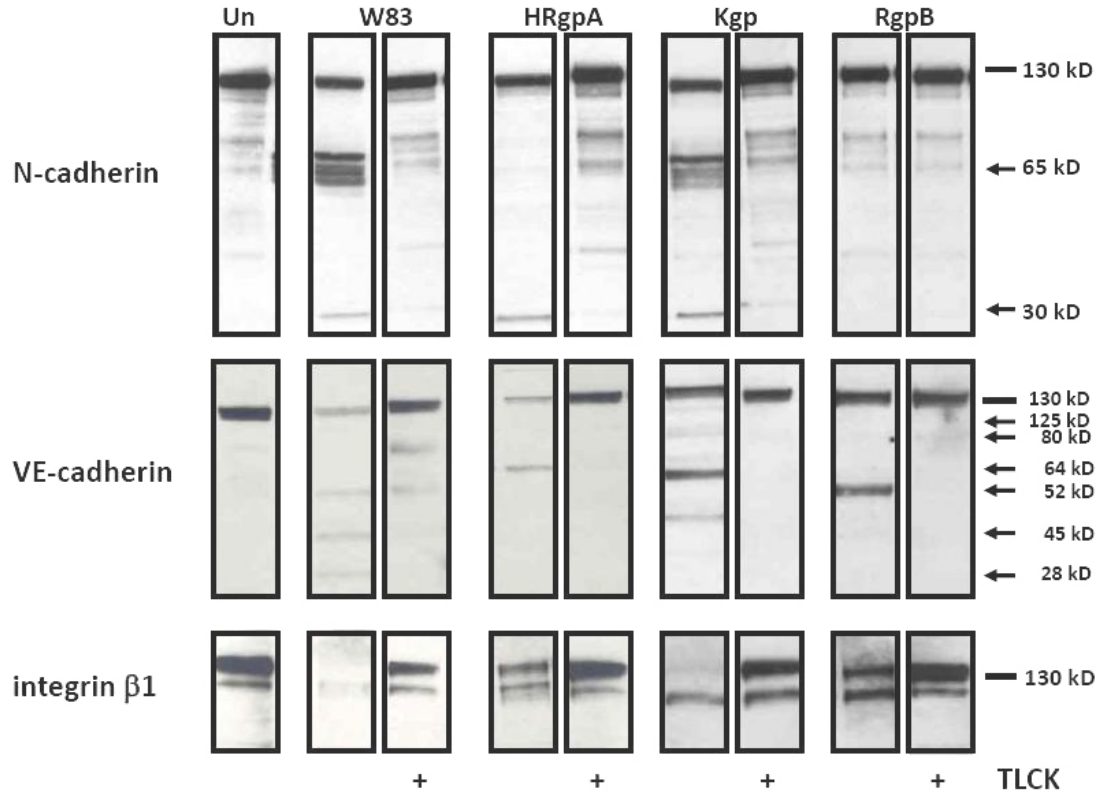


Figure 3. Synergism of purified gingipains in induction of CAM cleavage. 15 μ g of total protein from BCAEC treated for 6 h (N-cadherin), 24h (VE-cadherin), or 12h (integrin β 1), in the presence of 5 mM L-cysteine, with purified HRgpA (8 μ g/ml), Kgp (3 μ g/ml), or RgpB (5.2 μ g/ml) (all equivalent to 113 Units of Rgp activity/ml media or 12.4 Units of Kgp activity/ml media) were separated by SDS-PAGE and immunoblotted with antibodies to N-cadherin, VE-cadherin, and integrin β 1. Arrows indicate cleavage products, whereas lines indicate intact proteins. Un, untreated; +, present. Reproduced with permission from ASM (183).

than VE-cadherin cleavage (Figure 2A). These cleavages were confirmed *in vitro* by direct exposure of immunoprecipitated N- and VE-cadherin to gingipains (182). Using selective gingipain inhibitors, we demonstrated that Kgp activity was responsible for degradation of integrin β 1 while both Kgp and Rgp activity were responsible for cleavage of N-cadherin and VE-cadherin (183). In experiments were BCAEC were exposed to purified gingipains, Kgp was found to be the most efficient gingipain at cleaving integrin β 1 while N-cadherin was cleaved by both HRgpA and Kgp but not by RgpB (Figure 3). VE-cadherin was cleaved by HRgpA, Kgp, and RgpB, (Figure 3). BCAEC treated with purified Kgp reattached and remained viable during the time course but still exhibited cleaved/degraded CAMs, most notably integrin β 1 (Figure 3). This indicated that re-attachment was not because of the loss of Kgp activity.

Taken together, these results showed that the gingipains work together to detach cells and cleave specific CAMs (183). Although it cannot be ruled out that these observations may be cell type dependent, they implicate a role for gingipains in the pathogenesis of periodontitis and possibly modulation of host cell survival through detachment-induced apoptosis.

5.1.2. CAM degradation may implicate anoikis in gingipain-induced apoptosis

Anoikis is a type of caspase-dependent apoptosis that can be triggered by cellular detachment that is associated with loss of integrin survival signaling or inappropriate cell-matrix contacts (57,233). Anoikis has been described in numerous cell types, including epithelial cells (57), endothelial cells (127,166), keratinocytes, thyroid cells, fibroblasts, and osteoblasts (233).

Numerous kinase and phosphatase signaling molecules have been implicated as major regulators of anoikis [reviewed in (58)]. It has been reported that gingipains cleave focal adhesion kinase (FAK) and p130^{Cas} (79), which suggests the possibility that gingipains can induce anoikis in BCAEC. FAK acts as a bridge protein linking growth factor receptor and integrin stimulated signaling pathways and is potentially able to modulate anoikis because it is connected to signaling pathways involving the cytoskeleton and transcriptional activity in the nucleus (136). It has been shown that gingipains cleave FAK in immortalized human oral keratinocytes, which could be inhibited by peptide caspase inhibitors (79), suggesting Kgp involvement in this cleavage (183). The cleavage of FAK has several consequences related to

apoptosis induction. For example, loss of FAK signaling triggers Bid and Bax translocation (167). In addition, it was also reported that FAK interacts directly with receptor-interacting protein (RIP), which is a Fas receptor binding protein that induces apoptosis, and that the caspase cleavage fragment of FAK inhibits FAK survival signaling (167). This raises the possibility that a gingipain-induced FAK cleavage fragment may also be self inhibitory. Furthermore, the cleavage of FAK may disrupt its ability to be phosphorylated and thereby activating p53 and cell death (84). Further studies demonstrating that FAK is cleaved in BCAEC, determining whether Kgp is responsible for FAK cleavage, and exploring the inability of Kgp to induce apoptosis at the levels used in our models system need to be performed.

Viable BCAEC treated with purified Kgp displayed no full length integrin $\beta 1$, whereas detached apoptotic BCAEC treated with HRgpA or RgpB still had some full length protein (Figure 3). This would suggest that signaling pathways triggered by the loss of functional integrin $\beta 1$ do not play a role in gingipain-induced apoptosis. *P. gingivalis* activates the PI3-K/Akt pathway to increase host cell survival in response to apoptotic triggers (230), raising the possibility that in BCAEC treated with purified Kgp these same pathways are activated to prevent initiation of apoptosis in response to cell detachment. In addition, the cytosolic domain of integrin requires membrane localization to promote apoptosis, and it is possible that cleavage of the β integrin cytosolic domain observed in extracellular matrix (ECM)-deprived cells may even act to delay apoptosis by preventing integrin mediated death (196).

In intestinal epithelial cells, anoikis involved the activation of the initiator caspases-2 and -9 within minutes of detachment followed by the activation of caspases-7, -3, and -6, with caspase-8 activation occurring downstream of caspase-9 and -2 activation (70). Caspase-1 and -10 remained inactive (70). Caspase-8 activation occurred independently of downstream caspases or caspase-9 (70). Yet, the caspase-8 inhibitor z-IETD was also able to block anoikis, suggesting that at least two caspase pathways were activated for induction and execution of anoikis (70). By contrast, in gingival fibroblasts infected with *P. gingivalis*, caspases-3, -6, -7, -9, but not -8, -10, or -12, were activated which strongly points to a mitochondrial mediated apoptosis pathway (211). Further analysis of the hierarchy of caspase activation in BCAEC treated with gingipains will clarify the role that anoikis plays in gingipain-induced apoptosis.

5.2. *P. gingivalis*-induced apoptosis

5.2.1. Apoptosis can occur with and without caspase involvement

5.2.1.1. Caspase-dependent *P. gingivalis*-induced apoptosis

Because of the close proximity between oral pathogens and host cells in the gingiva, the interplay of *P. gingivalis* and the various cell types present in the gingival crevice has been under intense scrutiny. It is becoming increasingly clear that host cell responses to pathogenic

bacteria can involve either elevated apoptosis or suppression of cell death (144). The body of evidence reveals that *P. gingivalis*-induced apoptosis is cell type-specific.

Murine infection studies using live whole cells of *P. gingivalis* found that apoptosis is induced in fibroblasts and that it is modulated by TNF- α (67). Moreover, immortalized human keratinocytes infected with *P. gingivalis* rapidly exhibit significant levels of apoptosis (79). In order to ascertain the active constituent(s) in *P. gingivalis* that induces apoptosis, many have not used whole *P. gingivalis*, but rather different fractions of the bacterium or its culture fluid. Heat-stable molecules secreted in the culture medium of *P. gingivalis* induced apoptosis in peripheral blood mononuclear cells (63). In addition, studies in T-cells demonstrated that activation of caspase-3 by *P. gingivalis* culture fluid was not inhibited by protease inhibitors (76). Myocardial cells treated with *P. gingivalis* culture supernatant became apoptotic and had increased levels of Bad, calcineurin, a calmodulin-dependent serine/threonine phosphatase that is a mediator of cardiomyocyte hypertrophy and apoptosis, and the calcineurin downstream effector nuclear factor of activated T cells-3 (NFAT-3) (110). These results suggested that calcineurin dephosphorylates NFAT-3 and Bad, causing myocyte hypertrophy and triggering apoptosis (110). In addition, in this same system *P. gingivalis* culture supernatant induced DNA fragmentation and activation of caspases-3, -8, and -9. These effects could be blocked by inhibitors of p38 and ERK, but enhanced by a JNK inhibitor (111), suggesting that *P. gingivalis* signals through p38 and ERK to initiate apoptosis and that the JNK pathway may counteract this signaling.

One of the metabolites in *P. gingivalis* culture supernatant is butyric acid which induced calmodulin-sensitive apoptosis in two different B lymphoma cell lines (108). Butyric acid induced caspase-8 and -9 dependent apoptosis in T-cells that is independent of Fas (109). Another feature of the culture supernatant is that it can contain *P. gingivalis* cell membrane, which has been shown to induce apoptosis in T cells (64). *P. gingivalis* LPS has been demonstrated to induce apoptosis in the thymus, spleen, and lymph nodes in a mouse model (90). Culture supernatant can also include vesicles that contain both cell membrane and gingipains. In HeLa cells (human epithelial-like cells) treated with microspheres coated with *P. gingivalis* vesicles, a portion of the cells detached and displayed apoptotic morphology within 6 h, but detachment and cell death were not demonstrated with longer treatment or with gingipain-deficient mutants (88).

The relationship between gingipains and apoptosis has also been intensely studied for more than a decade. The first reports investigated the cytotoxicity of gingipains on different epithelial cell lines and found that culture supernatants with gingipain activity caused the cells to round up and detach from the monolayer concomitant with apoptotic morphology in some cell lines (181). Cultured human gingival fibroblasts also rounded up, detached, and lost cell viability when exposed to outer

membrane vesicles that possessed gingipain activity (137). However, low levels of cytotoxicity were induced in peripheral blood mononuclear cells by the gingipains (197).

Studies with gingival fibroblasts and epithelial cells treated with culture supernatants in the presence and absence of leupeptin, which inhibits Rgp activity, revealed that both Rgp and Kgp activities are responsible for cell detachment (93). More specific assays for apoptotic cells established that culture supernatants with gingipain activity were responsible for human gingival fibroblast detachment and apoptosis (223). Our group has also shown that gingipain active extracts induce epithelial cell detachment and apoptosis (34). Using extracts of wild type *P. gingivalis*, in the presence and absence of Rgp and/or Kgp inhibitors, along with Rgp and/or Kgp mutants, Baba and colleagues reported that both gingipains, especially Rgp, were responsible for cell detachment and loss of viability in human gingival fibroblasts (10) and endothelial cells (11). Our recent work extended these results by using purified gingipains to further elucidate the individual roles of the different gingipains in BCAEC apoptosis (182,183). Despite the ability of HRgpA, Kgp, and RgpB to cleave CAMs to varying degrees, only HRgpA and RgpB were able to induce apoptosis in BCAEC, with RgpB-induced apoptosis occurring slower (182,183). Unexpectedly, BCAEC treated with Kgp, after initially detaching and displaying cellular blebs, were able to re-adhere to the culture surface with no apparent loss of cell viability (182). In addition, detached cells produced by Kgp appear to still adhere to one another, whereas cells detached in the presence of HRgpA or RgpB subsequently died by apoptosis. The slower detachment and apoptosis induced by RgpB is not unexpected and may be explained by the absence of an adhesin domain. The adhesin domain of HRgpA, and most likely Kgp as well, provides a means of attachment to cells (32,33) through integrin $\beta 1$ (176) that RgpB does not have. Moreover, HRgpA has been shown to be more effective than RgpA_(cat) in decreasing the expression of integrin $\beta 1$ (176) because the adhesion domain enhances the binding of HRgpA to the cell.

N-cadherin signaling exerts an anti-apoptotic function by complexing with pro-caspase-8 which interferes with the recruitment of procaspase-8 to the DISC and initiation of apoptosis (74). Therefore, it is tempting to speculate that cleavage of N-cadherin, and the possible loss of its function, may be integral to the initiation of gingipain-induced apoptosis. In support of this, our results demonstrated that N-cadherin cleavage by HRgpA produced a single cleavage product of 30 kD (Figure 3). However, cleavage by Kgp generated fragments of approximately 65 kD and 30 kD and RgpB did not cleave N-cadherin within the time frame tested (Figure 3). These results suggest that cleavage of N-cadherin is not vital to the apoptotic trigger by gingipains since BCAEC treated with RgpB had no cleaved N-cadherin, but were apoptotic, and BCAEC treated with Kgp had cleaved N-cadherin, but were viable. As stated earlier, in this system Kgp was functional throughout the time course since it was able to cleave integrin $\beta 1$ at all time points through 24 h. A potential role for N-cadherin in gingipain induced BCAEC

apoptosis cannot be completely ruled out solely on the basis of its apparent lack of cleavage in apoptotic cells induced by treatment with RgpB. At 24 h there appeared to be decreased levels of N-cadherin cleavage by Kgp, as evidenced by the disappearance of some of the cleavage products in the 65kD group and the 30kD cleavage product (our data not shown). This suggests that BCAEC are in some way able to protect N-cadherin from cleavage by Kgp activity at later time points in order to counteract the apoptotic signaling and/or stimulate survival signaling. A similar phenomenon was observed in the gingipain-mediated cleavage of TNF- α in fibroblasts, in which HRgpA was shown to have the most activity toward TNF- α , but Kgp-induced cleavage of TNF- α was greatly increased if TNF- α re-expression was prevented, suggesting that the loss of TNF- α through Kgp cleavage could be compensated for by *de novo* synthesis (128), allowing cells to offset the Kgp-induced effects. Moreover, we have observed that a high concentration of Kgp can cause BCAEC detachment and apoptosis (our unpublished observations) suggesting that there is a threshold level of gingipain activity below which a cell can remain viable, but at gingipain activity above this level the cell cannot overcome gingipain-induced effects and becomes apoptotic. This threshold phenomenon has been demonstrated in epithelial cells treated with HRgpA (175).

Because of the differing abilities of the gingipains to cleave N-cadherin, VE-cadherin and integrin $\beta 1$, induce cell detachment, and apoptosis, it can be assumed that HRgpA, RgpB, and Kgp have other substrate specificities, which could explain the dramatic divergence in cell survival and death produced by these gingipains. It is conceivable that there may be differences in the ability of the specific gingipains to cleave one or more CAMs that may be pivotal for gingipain-induced apoptosis in BCAEC. Further investigation to define their substrate specificity and the apoptotic pathway components modulated by the purified gingipains will help pinpoint the nature of the Rgp-induced apoptotic trigger.

5.2.1.2. Caspase-independent *P. gingivalis* –induced apoptosis

The involvement of non-caspase proteases in some cases of apoptosis is suggested by observations that inhibition of caspases generally causes delays, but does not fully block cell death resulting from most apoptotic stimuli (94). While the ability of proteases to implement apoptotic signaling in the absence of caspase activity is not uncommon, our research work is the first to present initial evidence that HRgpA and RgpB can induce a caspase-3 and -7 independent apoptosis (183). The non-caspase proteases most closely associated with apoptosis are cathepsins, calpains, and granzymes (94). Cathepsins are translocated from lysosomes into the cytoplasm during apoptosis, where they intersect with and augment other apoptosis signaling mechanisms (94). Since HRgpA has been shown to rapidly traverse the plasma membrane (175), there is the possibility that the gingipains could interact with apoptotic signaling pathways in a manner similar to the cathepsins. Furthermore, HRgpA enters the nucleus (175) which may provide even more possibilities for

Gingipain-dependent host interactions

HRgpA and possibly Kgp, since catalytic activity was not required for protease translocation, to modulate host cell death and survival pathways.

Calpains are known to cleave many important proteins that are involved in cellular architecture which may be particularly important during apoptosis [reviewed in (94)]. Because of the ability of gingipains to cleave FAK, a substrate of both caspases (54) and calpains (94), paxillin, β -catenin, γ -catenin, p120, integrin β 4, Src, and p130^{Cas} (79) and as our work demonstrates other CAMs, such as N-cadherin, VE-cadherin, integrin β 1 (182,183), gingipains may have a similar role in apoptosis as calpains. Further similarities reside in the ability of calpains and gingipains to cleave pro-caspases, although the calpain-induced cleavage neither activates nor inactivates caspase-3 and -9 (94), whereas gingipain cleavage does activate procaspase-3 (211). Calpain and caspase-mediated apoptotic pathways can be inter-related (94) which raises a similar possibility for gingipains in light of our discovery of the caspase-independence of gingipain-induced apoptosis (183).

Because of the ability of granzyme A to cleave after lysine and arginine residues (91,124) and its ability to induce caspase-independent cell death (194), it is tempting to make a case that gingipains may behave similar to granzyme A. While granzyme A is the most abundant protease found in the granules of CTL cells it induces apoptosis after prolonged exposure making its role in apoptosis induction far more subtle than that of granzyme B (94). However, treatment of cells with granzyme A and perforin, a CTL granule protein that permeabilizes cell membranes, induces a caspase-independent, rapid accumulation of DNA strand breaks with subsequent nuclear condensation without the cleavage of poly(ADP-ribose) polymerase (PARP) and lamin B (94). However, there appears to be cell-type specific variance in some of the Granzyme A-induced apoptotic features (234). Granzyme A-induced cell death is marked by rapid induction of other apoptotic features, including loss of membrane integrity with blebbing, loss of mitochondrial membrane potential and production of ROS, chromatin condensation, and nuclear fragmentation (18,52,53). Mitochondrial cytochrome c release, oligonucleosomal DNA fragmentation, caspase activation, and Bcl-2 over-expression, all hallmarks of caspase-dependent apoptosis, are absent from granzyme A-induced apoptosis (52). Independent of caspases and Bcl-2, granzyme A cleaves lamins A, B, and C disrupting the nuclear lamina, a critical step in the progression of apoptosis (234). After gaining access to the nucleus (52,53), perhaps through the cleavage of lamins (234), granzyme A rapidly degrades histone H1 and the core histones disturbing the chromatin and facilitating exogenous DNase access to the DNA (235). In comparison, granzyme B/perforin-mediated apoptosis usually involves caspase activation, which granzyme B can initiate by the processing of pro-caspase-3, -7, and -9 to the active forms, and produces cleaved PARP and lamin B (94). However, granzyme A can also induce apoptotic features in a caspase-independent manner (94). A recent report showed that the cleavage of Bid by granzyme B

occurs at a residue distinct from the caspase cleavage site. This initiates apoptosis mediated by cytochrome c, SMAC release upstream of caspase activation, and loss of mitochondrial transmembrane potential

There are several commonalities between granzyme A and gingipains such as cleavage after lysine and arginine residues, translocation to the nucleus, and induction of caspase-independent cell death. However, the apoptotic features induced by gingipains needs to be further characterized. For example, it is unknown whether ssDNA nicks are produced during caspase-independent gingipain-induced apoptosis in BCAEC or if only oligosomal DNA fragmentation is produced in both caspase-dependent and -independent gingipain-induced apoptosis. The morphology of the nucleus and chromatin during both caspase-dependent and -independent gingipain-induced apoptosis in BCAEC needs to be carefully analyzed for more detailed classification of the type of cell death induced. In addition, the effects of gingipains on the mitochondria of BCAEC are completely unknown. It would also be interesting to ascertain what classical apoptotic signaling molecules are activated during both types of apoptosis triggered by gingipains in BCAEC. For example, granzyme A-induced apoptosis is not characterized by Bcl-2 upregulation, since it is caspase-independent (52). However, Bcl-2 can block caspase-independent apoptosis by preventing the release of cytochrome c in some systems (48) and can also prevent granzyme B-mediated apoptosis (208). It is tempting to speculate that Rgp activity could induce caspase-independent apoptosis via proteolytic activation of Bid at Arg⁶⁵, similar to cathepsins (208), with subsequent cytochrome c release and caspase-9 activation. A recent report demonstrated that *Clostridium difficile* Toxin-A-induced apoptosis is mediated by the cleavage of Bid prior to the activation of caspase-8, -9, -6, and -3 (28), so a bacterial mediated activation of Bid in the absence of active caspases is not improbable. We have summarized in Figure 4 a potential model of a granzyme-like mechanism for gingipain-induced caspase-independent apoptosis in BCAEC.

Gingipain activity may be responsible for caspase-like activity in cells (23) and it appears that gingipains may be able to cleave some of the same cellular substrates as caspases, such as FAK (79), N-cadherin (182,183), Topoisomerase I (Topo I) (182), and Lamin B (our unpublished observations). These results make it tempting to speculate that the gingipains can aid and possibly initiate apoptosis. A recent report described two independent apoptotic mechanisms simultaneously induced by a single stimulus. In the first pathway, there was breakdown of focal adhesion components leading to anoikis, while the second was a direct effect through receptor-mediated signal transduction pathways initiated by a cell adhesion domain (204). Conceivably, this scenario could be occurring during gingipain-induced apoptosis in BCAEC. Gingipains cleave many proteins involved in focal adhesion which can lead to anoikis. Secondly, the cleavage of CAMs by gingipains, such as N-cadherin which is involved in pro-caspase-8 recruitment to the DISC (74), may induce apoptosis concurrently. Additionally,

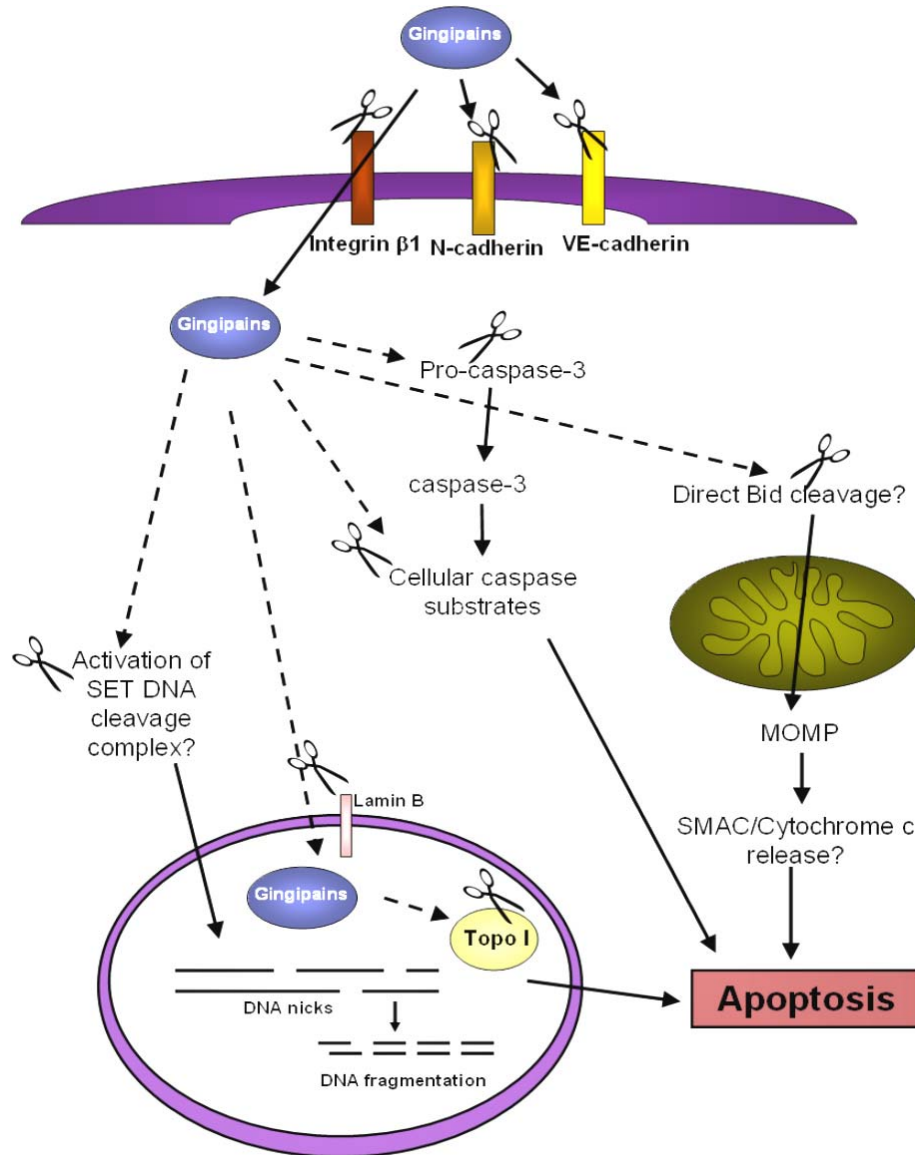


Figure 4. Potential granzyme-like model of gingipain-induced apoptosis. Through adhesion and cleavage of CAMs, most likely integrin $\beta 1$ (176), gingipains gain access to the host cell cytoplasm. In the cytoplasm gingipains may cleave pro-caspase-3 to the active caspase-3 (211) (as indicated by the scissors) that can cleave other cellular substrates, which may be cleaved by gingipains as well, to produce apoptotic morphology. It remains to be seen if gingipain activity could cleave Bid (208) (as indicated by the dashed arrow) producing MOMP, cytochrome c release, and apoptosis. Similar to granzyme A (19), gingipain activity could activate the SET complex that once inside the nucleus produces DNA nicks and fragmentation and apoptosis. Finally, it is conceivable that the possible degradation of lamin B by gingipain activity provides a means for gingipains to enter the nucleus and interact with nuclear proteins, such as Topo I, or allows escape of nuclear proteins to the cytoplasm where gingipains could cause their cleavage. Modified from (225) and (52).

Gingipains can signal a third pathway of cell death that is caspase-independent (183). This pathway is not caused by cell detachment since the broad caspase inhibitor z-VAD-FMK has been shown to block anoikis (170), and is not mediated by the JNK pathway since active caspases are needed for JNK to respond to the loss of cell-matrix contact (59).

Our studies demonstrated that gingipain-active W83 extracts, HRgpA, and RgpB induce apoptosis with a

more pronounced blebbing in the absence of active caspases (182). It will be interesting to determine if death-associated protein kinase (DAP kinase) as well as DAP kinase-related kinase (DRP kinase) are involved in this type of cell death. They are members of a family of calcium/calmodulin-regulated serine/threonine death kinases (72) that have been shown to be involved in membrane blebbing and function independent of caspase activity (238). Other proteins that may be activated by gingipains in the presence of z-VAD-FMK are p21-

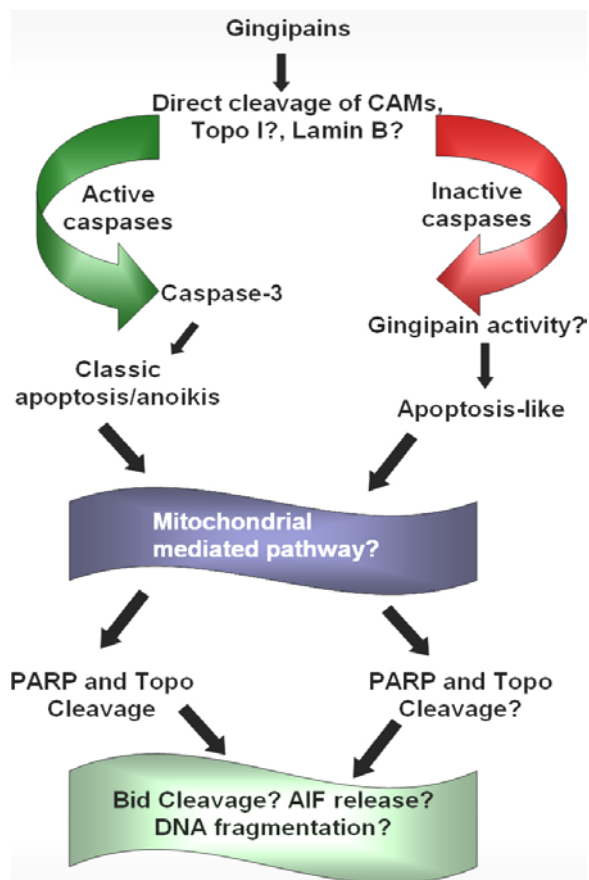


Figure 5. Comparison of potential caspase-dependent and -independent apoptosis pathway members induced by gingipain activity. Treatment of endothelial cells with gingipains causes the cleavage of CAMs and possibly Topo I and lamin B. In the presence of active caspases, this produces classically defined apoptosis and/or anoikis that is most likely mediated by the mitochondrial pathway of apoptosis (211). Apoptosis triggers the cleavage of PARP and Topo I. Other proteins that have yet to have their role defined in gingipain-induced apoptosis are Bid and AIF. In addition, it is unknown at this time whether DNA cleavage is a significant feature of gingipain-induced caspase-dependent apoptosis. Alternatively, the cleavage of CAMs and possibly Topo I and lamin B in the absence of active caspases, may provide the opportunity for gingipain-activity to initiate apoptosis that may also be mitochondrial in nature. The roles of PARP and Topo I cleavage in caspase-independent apoptosis, as well as, Bid, AIF, and DNA fragmentation need to be clarified.

activated kinase 2 or Rho-activated serine/threonine kinase, which also produce cellular blebbing (113).

Other features of caspase-independent cell death involve loss of mitochondrial membrane potential, cytochrome c and apoptosis-inducing factor (AIF) release from the mitochondria, zeiosis, phosphatidylserine translocation, chromatin cleavage to 50 kD pieces, and chromatin condensation and margination (228). While the nature of caspase-independent gingipain-induced apoptosis

in BCAEC needs to be further characterized, there are several proteins that maybe involved in this pathway that merit initial studies. For example, AIF was identified in human coronary artery endothelial cells outside the nucleus in the basal state and translocated to the nucleus upon induction of apoptosis in a caspase-independent manner (236). Moreover, treatment of BCAEC with gingipains may affect mitochondrial function (17), leading to the release AIF and/or endoG, both of which induce apoptotic morphology (89). Another possible protein involved in gingipain-induced caspase-independent signaling pathway could be Bax since it induces cytochrome c release that is not inhibited by z-VAD-FMK (9). *P. gingivalis* also modulates Bcl-2 levels (144) and Bcl-2 has been shown to block caspase-independent apoptosis via antioxidant effects (22). It will be interesting to compare the modulation of Bcl-2 in gingipain-induced caspase-dependent and -independent apoptosis.

Besides apoptosis-related proteins AIF, Bax and Bcl-2, gingipains may modulate other proteins to activate caspase-independent signaling. Interestingly, Topo I, a substrate for both caspases and cathepsins, was cleaved to a 45 kD fragment in the presence of active gingipains (182,183). A fragment of this size usually appears in caspase-independent, necrotic cell death and is generated by cathepsins (154), however, we did not observe necrosis during gingipain-induced cell death in BCAEC. This suggests that gingipains might be able to directly cleave Topo I. This pattern of Topo I cleavage was observed in BCAEC cells exposed to purified gingipains or gingipain-active W83 extracts in the presence and absence of selective inhibitors of Rgp and Kgp (183), suggesting that both Rgp and Kgp can cleave Topo I to varying degrees. However, Topo I cleavage may not be relevant to gingipain-induced apoptosis since endothelial cells treated with Kgp that are attached and viable also display this 45 kD cleavage fragment.

Lamins are major structural proteins of the nuclear envelope (37) that are cleaved by caspases to induce chromatin condensation (207) and possibly disrupt lamin-lamin interactions, as well as, interactions of lamins with other nuclear components (37). Cleavage of lamins also results in nuclear shrinkage (113). HRgpA localizes to the nucleus (175) and may be able to cleave lamin B and subsequently gain access to the nucleus to cleave Topo I and possibly modulate other nuclear proteins, producing the nuclear apoptotic morphology. Kgp may function similarly to and work with HRgpA, since its nuclear targeting ability was not dependent on proteolytic activity (175). Figure 5 is a comparison of potential caspase-independent apoptosis pathway members induced by gingipain activity.

5.3. *P. gingivalis*-induced resistance to apoptosis

Despite several reports of the ability of *P. gingivalis* whole cells, culture supernatants, membranes, and gingipains to induce apoptosis, there is also data indicating that *P. gingivalis* inhibits apoptosis in some cell types. Furthermore, there is evidence that *P. gingivalis* infection may initiate divergent survival and death pathways (36). *P. gingivalis* fimbriae inhibited growth

factor deprivation-induced apoptosis via ERK-dependent expression of p21 in a human monocytic cell line (153). In addition, it has been demonstrated that *P. gingivalis* LPS can prevent apoptosis of neutrophils derived from a human promyelocytic cell line, which may prolong an acute inflammatory response resulting in an increased potential for tissue destruction (140). Experimental results of Nakhjiri, *et al.*, suggested that the first response to *P. gingivalis* infection in primary gingival epithelial cells was apoptosis; however, over time *P. gingivalis* initiated anti-apoptotic signaling via increased levels of Bcl-2 and decreased levels of Bax that canceled the cells' death response and was even able to promote cell survival in the presence of the apoptotic inducer camptothecin (144). Furthermore, inhibition of the PI3-K/Akt pathway abolished the *P. gingivalis*-induced protection from apoptosis (230). We believe that these studies demonstrated that apoptosis was induced first since a reversible phosphatidylserine exposure triggered by *P. gingivalis* invasion could be inhibited by z-VAD-FMK (230), suggesting that caspases are activated but the anti-apoptotic signaling overrides the apoptotic signaling. Yilmaz, *et al.* proposed that *P. gingivalis* inhibited mitochondrial-dependent apoptosis in gingival epithelial cells in order to maintain its intracellular lifestyle long enough to allow the successful spread of the bacteria to adjacent cells (231) and deeper host tissues (5,230). In contrast, another report found that heat-killed *P. gingivalis* induced caspase-3 and -8 activation and apoptosis in gingival epithelial cells through FasL-mediated apoptosis that was mediated by nuclear factor- κ B (NF- κ B) and Toll-like receptor -2 (TLR-2) (25). Brozovic, *et al.* surmised that NF- κ B activation was likely to be both anti-apoptotic and pro-apoptotic, depending on the stimulus, such as variances in bacterial concentrations, and the specific cell type involved (25).

Evidence is emerging that *P. gingivalis* can modulate several different monocytic cell survival mechanisms in order to evade host defenses such as, signal transducer and activator of transcription 6 (STAT6), which permits nitric oxide synthesis in macrophages; interferon-dependent positive-acting transcription factor (STAT1), which is essential in caspase-independent cell death of activated macrophages; double stranded RNA (dsRNA) - activated protein kinase (PRKR), which may be important in the anti-apoptotic signaling by *P. gingivalis*; calreticulin precursor (CALR), which binds complement C1q modulating the host immune response, and gene associated with retinoid-interferon induced mortality (GRIM19) (237). GRIM19 appears to be a nuclear protein that is expressed in response to interferon and retinoic acid, induces cell death (7), and is involved in pathogen recognition and invasion (13). Moreover, in human immortalized gingival keratinocytes *P. gingivalis* modulated 25 apoptosis-associated genes linked to p53 and the mitochondrial pathway of apoptosis (75). A recent study defined the anti-apoptotic pathways mediated by *P. gingivalis* in response to camptothecin treatment. It was demonstrated that *P. gingivalis* transiently activates the JAK1/Stat3 pathways with Akt pathway modulation also being involved. These pathways up-regulate Survivin, at the mRNA level, and

ultimately block activation of caspase-3 (122). Considering the many diverse signaling pathways engaged in apoptosis, it is not surprising that some bacteria inhibit or delay apoptosis while others induce it (230).

Interestingly, *P. gingivalis* can recognize different host cell types and is capable of targeting specific and distinct signaling pathways (6). For example, *P. gingivalis* has been shown to be uniquely capable of selectively activating one MAPK pathway and down-regulating another which may cause responses of epithelial cells to be species and even strain specific (224). In addition, the effects that are produced in a particular host cell may be specific to the infecting bacterial strain. Out of 15 clinical isolates and a reference American Type Culture Collection (ATCC) strain 33277 of *P. gingivalis* only one strain was cytotoxic to KB epithelial cells (49). Interestingly, the strains used in this study had a higher lysine-specific cysteine protease activity than the reference strain and there was no decrease in arginine-specific activity in strains that did not possess an *rgpB* gene (49). These results suggest that not only may the effects triggered be dependent on the host cell, but may also be related to the strain of *P. gingivalis* used, especially since our group has shown that gingipain-active extracts from strain W83 induced apoptosis in KB cells (34). *P. gingivalis* has also exhibited the ability to modulate the NF- κ B pathway differently during the course of infection. In gingival fibroblasts early NF- κ B activation occurred with a later decrease in activity via a PI3-K mediated pathway that appeared not to be mediated by the gingipains (34,211). It may be possible that the anti-apoptotic effects of *P. gingivalis* infection in some cell types signal initially through NF- κ B similar to *Rickettsia rickettsii*, whose primary target is vascular endothelial cells (227).

The recent findings that fimbriae from *P. gingivalis* modulated both pro-apoptotic and anti-apoptotic genes in human aortic endothelial cells (36) and in gingival fibroblasts (211) confirmed that *P. gingivalis* could induce either apoptosis or resistance to apoptosis. It appears that the pathway that prevails in a given cell type depends on the balance of cell death and survival modulators that are present. These results raise some questions. In cells in which *P. gingivalis* can induce apoptosis, is this because of direct pro-apoptotic signaling by *P. gingivalis* or because of a specific survival signal that is absent in those cells and is essential to prevent *P. gingivalis*-induced apoptosis? For instance, a recent report demonstrated that the same apoptotic stimulus induced an intrinsic apoptosis that was caspase-dependent in one cell type and caspase-independent in a different cell type (89), implying cellular differences and not apoptotic stimulus differences. In cells that survive despite *P. gingivalis* infection, is this because *P. gingivalis* is overriding the host cell's apoptotic reflex or because the cell is 'ambivalent' to the presence of the invading bacterium? It has been proposed that cells respond to changes that bacteria cause by activating the apoptotic mechanism, although they are not acting primarily to cause apoptosis (218).

There is overwhelming evidence that host cell responses to pathogenic bacteria can involve either apoptosis induction or suppression of cell death (144) and that host cells appear to be able to distinguish between infecting organisms and their components (237). Recently, it has been proposed that the possibly finite number of pathways that the pathogen may have evolved to modulate could be characteristic of the organism's genus (75). This suggests that the outcome of infection may be more determined by the presence or absence of the host cells' repertoire of defensive molecules than by components of the pathogen's virulence arsenal. A review of the literature indicates that *P. gingivalis* induces apoptosis in only some cell types. The molecules involved in triggering apoptosis by *P. gingivalis* are only beginning to be elucidated. It will be interesting to determine if the same molecules are involved in both gingipain-induced apoptosis and gingipain-induced resistance to apoptosis and to compare the morphological characteristics and pathways of caspase-dependent and -independent apoptosis mediated by the gingipains.

5.4. The purpose of pathogen-induced effects on host cell survival

Numerous microbes are known to affect host cell survival; some trigger apoptosis while others provide increased cell survival in host cells [reviewed in (61,62,66,138,227)]. Overall, there appears to be two general mechanisms for Gram-negative pathogen-induced cell death. In the first, effector proteins delivered by a Type III secretion system activate caspase-1 producing both features of apoptosis and necrosis. In the second mechanism, activation of TLR-4 by LPS triggers apoptosis (73).

Apoptosis induced in any host cell type by bacteria acting external to the cell is likely to be a pathogenic mechanism for promoting invasiveness (38). It would be beneficial to the bacteria to induce apoptosis of the host cell in order to evade the immune system by eliminating key defense cells like lymphocytes, monocytes, and neutrophils (227); however, cell death may also be a protective mechanism signaled by the host cell to induce inflammation through caspase-1 activation (61,62), clear the pathogen, and limit intracellular replication (38). For example, apoptosis of lung epithelial cells upon infection with *Pseudomonas aeruginosa* constitutes a pivotal part of host defense against *P. aeruginosa* infections. However, this apoptosis crucially contributes to the host defense against the bacteria (66). It is also possible that apoptosis contributes to the spread of intracellular bacteria, as appears to be the case for *Salmonella*-induced apoptosis of macrophages (62,135) through successive rounds of intracellular growth, apoptosis, and phagocytosis of infected apoptotic bodies, perpetuating the infection and ensuring cell to cell spread (73). In addition, apoptosis may play a role in bacterial trafficking into a niche permissive for intracellular replication (62), as in the case of *Shigella flexneri*, which kills macrophages by apoptosis and thereby gains access to Peyer patches, permitting the bacteria to spread and to infect enterocytes from the basolateral side (66). A recent report speculated that *P. gingivalis* invaded

HeLa cells and did not cause cell death to acquire intracellular persistence, but if the invasion is rather quick there was probably not enough time for complete *P. gingivalis*-induced anti-apoptotic signaling to initiate before pro-apoptotic signals induced some apoptotic morphology (88). However, it has also been suggested that an initial delay of apoptosis would allow *P. gingivalis* extra time to replicate intracellularly to a higher yield while providing protection from the host immune system before inducing apoptosis for bacterial spread to other cells (211). Anoikis could be triggered to prevent the lateral spread of bacteria and aid in the removal of the infected host cell (185). Collectively, these findings suggest that there is no universal function for pathogen regulated cell death (138).

Host cells infected by some pathogens may actually have increased cell survival that aids in pathogenesis. Inhibiting host cell apoptosis would provide a safe haven for an intracellular pathogen (227) and help to maintain the metabolic activity of the infected cell (62). For example, inhibiting host cell death facilitates the intracellular development of *Toxoplasma gondii*, which increases parasitemia and may lead to an enhanced inflammatory response to the parasite resulting in host mortality (120). Experimental evidence suggests that *T. gondii* inhibits apoptosis of host cells by different mechanisms, both direct inhibition of infected cells and indirect mechanisms, to protect both infected and uninfected host cells (120). Direct mechanisms interfere with caspase cascade activation, increase levels of anti-apoptotic Bcl-2 family members, increase inhibitor of apoptosis proteins (IAPs), and decrease levels of PARP that may inhibit apoptosis in a caspase-independent fashion (120). Remarkably, a single viable parasite was sufficient to block apoptosis of the host cell and this inhibition was reversed when the parasite was killed (120). Some pathogens signal both cell death and cell survival. For example, *Mycobacterium tuberculosis* induces apoptosis in macrophages via a TNF- α and caspase-1 dependent pathway, but also protects cells from apoptosis via NF- κ B and neutralization of TNF- α pro-apoptotic activity (62). Chronic infection with *Helicobacter pylori* is associated with gastric cancer suggesting that this bacterium would induce apoptosis resistance in gastric cells. However, it induces apoptosis in the majority of gastric cells, but a small percentage of cells are resistant to *H. pylori*-induced apoptosis, as well as, other apoptotic stimuli (187). The bacterial-related factors responsible for this effect are unclear. Furthermore, *Chlamydia* induces resistance to apoptosis during the initial stages of infection and then later induces apoptosis in host cells (227). This indicates that intracellular bacteria can fine tune the balance between anti- and pro-apoptotic activities even at discrete stages of infection (62), with the ultimate goal to facilitate the spread of infection.

6. SUMMARY

In order for *P. gingivalis* to survive and grow in the host, it must be able to obtain nutrients and overcome lethal factors, such as oxidative stress which may result from the host's immune response. The gingipains of *P.*

gingivalis are vital for the survival of the bacterium. They degrade host tissue to provide a source of nutrients and through degradation of cell adhesion molecules, induce apoptosis and access deeper tissue layers evading some aspects of the host immune system. Gingipains are involved in the accumulation of heme on the bacterial surface creating a shield from ROS, as well as, interacting with other gene products that are involved in oxidative stress resistance.

In summary, based on our observations, we can postulate a tentative model, which may be part of the mechanism for *P. gingivalis* virulence. Central to this model are the gingipains and the regulatory role of the *vim* gene products. 1) The accumulation of the earliest colonizers on the tooth surface initiates an inflammatory reaction known as gingivitis. 2) As the ecological succession continues, conditions in the subgingival area will facilitate the colonization of the anaerobes, in particular, *P. gingivalis*. Gingipains and the expression of the genes in the *recA* region in this organism are an important strategy in an environment with increasing inflammation and neutrophil infiltration. These gene products are required to facilitate survival of *P. gingivalis* in this oxidative stress environment. 3) The *recA* gene product is involved in repair of DNA damage while the products of the *vim* genes facilitate the appropriate production and cell-associated localization of the gingipains. The cell-associated localization of the gingipains may be important because protection from oxidative damage may also be facilitated by surface accumulation of heme which will form μ -oxo dimers in the presence of oxygen and its toxic reactive derivatives (190,193). *P. gingivalis* gingipains are known to be associated with heme accumulation [reviewed in (152)]. 4) Survival in the oxidative environment requires that *P. gingivalis* also utilize a combination of other mechanisms for growth and proliferation. Other mechanisms utilized by the bacterium such as Hydrogen peroxide reductase and Bcp can guard against oxidative damage which would curtail the growth of *P. gingivalis*. Gingipain-associated degradation of cell-cell adhesion proteins (cadherins and integrin) on epithelial and endothelial cells resulting in cell death will compromise tissue integrity. Increased tissue permeability will facilitate access of *P. gingivalis* to the vascular system, giving rise to other systemic effects (e.g. cardiovascular disease) potentially providing a more hospitable niche. Degradation of host tissues also provides nutrients for the organism. Since the mouth reflects the health of the whole body, as suggested by the Surgeon General (212), preventing gingipain activity in the oral cavity and, thus, inhibiting the key mediator of survival for *P. gingivalis* may be a means to greatly improve the overall health status.

7. ACKNOWLEDGMENTS

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8. REFERENCES

1. Abaibou, H., Z. Chen, G. J. Olango, Y. Liu, J. Edwards, and H. M. Fletcher: *vimA* gene downstream of *recA* is involved in virulence modulation in *Porphyromonas gingivalis* W83. *Infect Immun* 69 (1), 325 -335 (2001)
2. Amano, A., T. Ishimoto, H. Tamagawa, and S. Shizukuishi: Role of superoxide dismutase in resistance of *Porphyromonas gingivalis* to killing by polymorphonuclear leukocytes. *Infect Immun* 60 (2), 712 -714 (1992)
3. Amano, A., M. Kuboniwa, K. Kataoka, K. Tazaki, E. Inoshita, H. Nagata, H. Tamagawa, and S. Shizukuishi: Binding of hemoglobin by *Porphyromonas gingivalis*. *FEMS Microbiol Lett* 134 (1), 63 -67 (1995)
4. Amano, A., H. Tamagawa, S. Shizukuishi, and A. Tsunemitsu: Superoxide dismutase, catalase and peroxidases in oral anaerobic bacteria. *J Osaka Univ Dent Sch* 26 187 -192 (1986)
5. Andrian, E., D. Grenier, and M. Rouabhia: *In vitro* models of tissue penetration and destruction by *Porphyromonas gingivalis*. *Infect Immun* 72 (8), 4689 - 4698 (2004)
6. Andrian, E., D. Grenier, and M. Rouabhia: *Porphyromonas gingivalis*-Epithelial Cell Interactions in Periodontitis. *J Dent Res* 85 (5), 392 -403 (2006)
7. Angell, J. E., D. J. Lindner, P. S. Shapiro, E. R. Hofmann, and D. V. Kalvakolanu: Identification of GRIM-19, a novel cell death-regulatory gene induced by the interferon-beta and retinoic acid combination, using a genetic approach. *J Biol Chem* 275 (43), 33416 -33426 (2000)
8. Araki, M., K. Hiratsuka, M. Kiyama-Kishikawa, and Y. Abiko: Monitoring of *dnak* gene expression in *Porphyromonas gingivalis* by oxygen stress using DNA microarray. *J Oral Sci* 46 (2), 93 -100 (2004)
9. Arnoult, D., M. Karbowski, and R. J. Youle: Caspase inhibition prevents the mitochondrial release of apoptosis-inducing factor. *Cell Death Differ* 10 (7), 845 -849 (2003)
10. Baba, A., N. Abe, T. Kadowaki, H. Nakanishi, M. Ohishi, T. Asao, and K. Yamamoto: Arg-gingipain is responsible for the degradation of cell adhesion molecules of human gingival fibroblasts and their death induced by *Porphyromonas gingivalis*. *Biol Chem* 382 (5), 817 -824 (2001)
11. Baba, A., T. Kadowaki, T. Asao, and K. Yamamoto: Roles for Arg- and Lys-gingipains in the disruption of cytokine responses and loss of viability of human endothelial cells by *Porphyromonas gingivalis* infection. *Biol Chem* 383 (7-8), 1223 -1230 (2002)
12. Baillon, M. L., A. H. van Vliet, J. M. Ketley, C. Constantinidou, and C. W. Penn: An iron-regulated alkyl hydroperoxide reductase (AhpC) confers aerotolerance and oxidative stress resistance to the microaerophilic pathogen *Campylobacter jejuni*. *J Bacteriol* 181 (16), 4798 -4804 (1999)
13. Barnich, N., T. Hisamatsu, J. E. Aguirre, R. Xavier, H. C. Reinecker, and D. K. Podolsky: GRIM-19 interacts with nucleotide oligomerization domain 2 and serves as downstream effector of anti-bacterial function in intestinal epithelial cells. *J Biol Chem* 280 (19), 19021 -19026 (2005)

14. Bate, C. and A. Williams: Role of glycosylphosphatidylinositols in the activation of phospholipase A2 and the neurotoxicity of prions. *J Gen Virol* 85 (Pt 12), 3797-3804 (2004)
15. Batty, D. P. and R. D. Wood: Damage recognition in nucleotide excision repair of DNA. *Gene* 241 (2), 193-204 (2000)
16. Beaman, L. and B. L. Beaman: The role of oxygen and its derivatives in microbial pathogenesis and host defense. *Annu Rev Microbiol* 38 27-48 (1984)
17. Belton, C. M., P. C. Goodwin, S. Fatherazi, M. M. Schubert, R. J. Lamont, and K. T. Izutsu: Calcium oscillations in gingival epithelial cells infected with *Porphyromonas gingivalis*. *Microbes Infect* 6 (5), 440-447 (2004)
18. Beresford, P. J., Z. Xia, A. H. Greenberg, and J. Lieberman: Granzyme A loading induces rapid cytolysis and a novel form of DNA damage independently of caspase activation. *Immunity* 10 (5), 585-594 (1999)
19. Beresford, P. J., D. Zhang, D. Y. Oh, Z. Fan, E. L. Greer, M. L. Russo, M. Jaju, and J. Lieberman: Granzyme A activates an endoplasmic reticulum-associated caspase-independent nuclease to induce single-stranded DNA nicks. *J Biol Chem* 276 (46), 43285-43293 (2001)
20. Bhogal, P. S., N. Slakeski, and E. C. Reynolds: A cell-associated protein complex of *Porphyromonas gingivalis* W50 composed of Arg- and Lys-specific cysteine proteinases and adhesins. *Microbiology* 143 (Pt 7) 2485-2495 (1997)
21. Boiteux, S. and J. P. Radicella: Base excision repair of 8-hydroxyguanine protects DNA from endogenous oxidative stress. *Biochimie* 81 (1-2), 59-67 (1999)
22. Borner, C. and L. Monney: Apoptosis without caspases: an inefficient molecular guillotine? *Cell Death Differ* 6 (6), 497-507 (1999)
23. Boyce, M., A. Degterev, and J. Yuan: Caspases: an ancient cellular sword of Damocles. *Cell Death Differ* 11 (1), 29-37 (2004)
24. Brochu, V., D. Grenier, K. Nakayama, and D. Mayrand: Acquisition of iron from human transferrin by *Porphyromonas gingivalis*: a role for Arg- and Lys-gingipain activities. *Oral Microbiol Immunol* 16 (2), 79-87 (2001)
25. Brozovic, S., R. Sahoo, S. Barve, H. Shiba, S. Uriarte, R. S. Blumberg, and D. F. Kinane: *Porphyromonas gingivalis* enhances FasL expression via up-regulation of NFkappaB-mediated gene transcription and induces apoptotic cell death in human gingival epithelial cells. *Microbiology* 152 (Pt 3), 797-806 (2006)
26. Campbell, J. A., G. J. Davies, V. Bulone, V. and B. Henrissat: A classification of nucleotide-diphospho-sugar glycosyltransferases based on amino acid sequence similarities. *Biochem J* 329 (Pt 3) 719- (1998)
27. Canakci, C. F., Y. Cicek, and V. Canakci: Reactive oxygen species and human inflammatory periodontal diseases. *Biochemistry (Mosc)* 70 (6), 619-628 (2005)
28. Carneiro, B. A., J. Fujii, G. A. Brito, C. Alcantara, R. B. Oria, A. A. Lima, T. Obrig, and R. L. Guerrant: Caspase and bid involvement in *Clostridium difficile* toxin A-induced apoptosis and modulation of toxin A effects by glutamine and alanyl-glutamine *in vivo* and *in vitro*. *Infect Immun* 74 (1), 81-87 (2006)
29. Chapple, I. L. and J. B. Matthews: The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol* 2000 43 160-232 (2007)
30. Chapple, I. L. C.: Role of free radicals and antioxidants in the pathogenesis of the inflammatory periodontal diseases. *J Clin Pathol Clin Mol Pathol* 49 (5), M247-M255 (1996)
31. Chen, T., H. Dong, R. Yong, and M. J. Duncan: Pleiotropic pigmentation mutants of *Porphyromonas gingivalis*. *Microb Pathog* 28 (4), 235-247 (2000)
32. Chen, T. and M. J. Duncan: Gingipain adhesin domains mediate *Porphyromonas gingivalis* adherence to epithelial cells. *Microb Pathog* 36 (4), 205-209 (2004)
33. Chen, T., K. Nakayama, L. Belliveau, and M. J. Duncan: *Porphyromonas gingivalis* gingipains and adhesion to epithelial cells. *Infect Immun* 69 (5), 3048-3056 (2001)
34. Chen, Z., C. A. Casiano, and H. M. Fletcher: Protease-active extracellular protein preparations from *Porphyromonas gingivalis* W83 induce N-cadherin proteolysis, loss of cell adhesion and apoptosis in human epithelial cells. *J Periodontol* 72 641-650 (2001)
35. Choi, J. I., N. Takahashi, T. Kato, and H. K. Kuramitsu: Isolation, expression, and nucleotide sequence of the *sod* gene from *Porphyromonas gingivalis*. *Infect Immun* 59 (4), 1564-1566 (1991)
36. Chou, H. H., H. Yumoto, M. Davey, Y. Takahashi, T. Miyamoto, F. C. Gibson, III, and C. A. Genco: *Porphyromonas gingivalis* fimbria-dependent activation of inflammatory genes in human aortic endothelial cells. *Infect Immun* 73 (9), 5367-5378 (2005)
37. Cohen, G. M.: Caspases: the executioners of apoptosis. *Biochem J* 326 (Pt 1) 1-16 (1997)
38. Colino, J. and C. M. Snapper: Two distinct mechanisms for induction of dendritic cell apoptosis in response to intact *Streptococcus pneumoniae*. *J Immunol* 171 (5), 2354-2365 (2003)
39. Corcoran, M. L., D. E. Kleiner, Jr., and W. G. Stetler-Stevenson: Regulation of matrix metalloproteinases during extracellular matrix turnover. *Adv Exp Med Biol* 385 151-159 (1995)
40. Curtis, M. A., H. K. Kuramitsu, M. Lantz, F. L. Macrina, K. Nakayama, J. Potempa, E. C. Reynolds, and J. Aduse-Opoku: Molecular genetics and nomenclature of proteases of *Porphyromonas gingivalis*. *J Periodont Res* 34 (8), 464-472 (1999)
41. Curtis, M. A., A. Thickett, J. M. Slaney, M. Rangarajan, J. Aduse-Opoku, P. Shepherd, N. Paramonov, and E. F. Hounsell: Variable carbohydrate modifications to the catalytic chains of the RgpA and RgpB proteases of *Porphyromonas gingivalis* W50. *Infect Immun* 67 (8), 3816-3823 (1999)
42. Dashper, S. G., K. J. Cross, N. Slakeski, P. Lissel, P. Aulakh, C. Moore, and E. C. Reynolds: Hemoglobin hydrolysis and heme acquisition by *Porphyromonas gingivalis*. *Oral Microbiol Immunol* 19 (1), 50-56 (2004)
43. Demmer, R. T. and M. Desvarieux: Periodontal infections and cardiovascular disease: the heart of the matter. *J Am Dent Assoc* 137 Suppl 14S-20S (2006)
44. Demple, B. and L. Harrison: Repair of oxidative damage to DNA: enzymology and biology. *Annu Rev Biochem* 63 915-948 (1994)

45. Diaz, P. I. and A. H. Rogers: The effect of oxygen on the growth and physiology of *Porphyromonas gingivalis*. *Oral Microbiol Immunol* 19 (2), 88 -94 (2004)
46. Diaz, P. I., N. Slakeski, E. C. Reynolds, R. Morona, A. H. Rogers, and P. E. Kolenbrander: Role of oxyR in the oral anaerobe *Porphyromonas gingivalis*. *J Bacteriol* 188 (7), 2454 -2462 (2006)
47. Diaz, P. I., P. S. Zilm, V. Wasinger, G. L. Corthals, and A. H. Rogers: Studies on NADH oxidase and alkyl hydroperoxide reductase produced by *Porphyromonas gingivalis*. *Oral Microbiol Immunol* 19 (3), 137 -143 (2004)
48. Egger, L., J. Schneider, C. Rheme, M. Tapernoux, J. Hacki, and C. Borner: Serine proteases mediate apoptosis-like cell death and phagocytosis under caspase-inhibiting conditions. *Cell Death Differ* 10 (10), 1188 -1203 (2003)
49. Eick, S., J. Rodel, J. W. Einax, and W. Pfister: Interaction of *Porphyromonas gingivalis* with KB cells: comparison of different clinical isolates. *Oral Microbiol Immunol* 17 (4), 201 -208 (2002)
50. Eley, B. M. and S. W. Cox: Proteolytic and hydrolytic enzymes from putative periodontal pathogens: characterization, molecular genetics, effects on host defenses and tissues and detection in gingival crevice fluid. *Periodontol* 2000 31 105 -124 (2003)
51. Elkins, J. G., D. J. Hassett, P. S. Stewart, H. P. Schweizer, and T. R. McDermott: Protective role of catalase in *Pseudomonas aeruginosa* biofilm resistance to hydrogen peroxide. *Appl Environ Microbiol* 65 (10), 4594 -4600 (1999)
52. Fan, Z., P. J. Beresford, D. Y. Oh, D. Zhang, and J. Lieberman: Tumor suppressor NM23-H1 is a granzyme A-activated DNase during CTL-mediated apoptosis, and the nucleosome assembly protein SET is its inhibitor. *Cell* 112 (5), 659 -672 (2003)
53. Fan, Z., P. J. Beresford, D. Zhang, Z. Xu, C. D. Novina, A. Yoshida, Y. Pommier, and J. Lieberman: Cleaving the oxidative repair protein Ape1 enhances cell death mediated by granzyme A. *Nat Immunol* 4 (2), 145 -153 (2003)
54. Fischer, U., R. U. Janicke, and K. Schulze-Osthoff: Many cuts to ruin: a comprehensive update of caspase substrates. *Cell Death Differ* 10 (1), 76 -100 (2003)
55. Fletcher, H. M., R. M. Morgan, and F. L. Macrina: Nucleotide sequence of *Porphyromonas gingivalis* W83 *recA* homolog and construction of a *recA*-deficient mutant. *Infect Immun* 65 (11), 4592 -4597 (1997)
56. Forng, R. Y., C. Champagne, W. Simpson, and C. A. Genco: Environmental cues and gene expression in *Porphyromonas gingivalis* and *Actinobacillus actinomycescomitans*. *Oral Dis* 6 (6), 351 -365 (2000)
57. Frisch, S. M. and H. Francis: Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol* 124 (4), 619 -626 (1994)
58. Frisch, S. M. and R. A. Screaton: Anoikis mechanisms. *Curr Opin Cell Biol* 13 (5), 555 -562 (2001)
59. Frisch, S. M., K. Vuori, D. Kelaïta, and S. Sicks: A role for Jun-N-terminal kinase in anoikis; suppression by bcl-2 and crmA. *J Cell Biol* 135 (5), 1377 -1382 (1996)
60. Gallagher, A., J. Aduse-Opoku, M. Rangarajan, J. M. Slaney, and M. A. Curtis: Glycosylation of the Arg-gingipains of *Porphyromonas gingivalis* and comparison with glycoconjugate structure and synthesis in other bacteria. *Curr Protein Pept Sci* 4 (6), 427 -441 (2003)
61. Gao, L. and K. Y. Abu: Hijacking of apoptotic pathways by bacterial pathogens. *Microbes Infect* 2 (14), 1705 -1719 (2000)
62. Gao, L. Y. and Y. A. Kwaik: The modulation of host cell apoptosis by intracellular bacterial pathogens. *Trends Microbiol* 8 (7), 306 -313 (2000)
63. Geatch, D. R., J. I. Harris, P. A. Heasman, and J. J. Taylor: *In vitro* studies of lymphocyte apoptosis induced by the periodontal pathogen *Porphyromonas gingivalis*. *J Periodont Res* 34 (2), 70 -78 (1999)
64. Gemmell, E., S. Prajane, D. A. Grieco, J. J. Taylor, and G. J. Seymour: Apoptosis in *Porphyromonas gingivalis*-specific T-cell lines. *Oral Microbiol Immunol* 14 (6), 331 -338 (1999)
65. Genco, C. A. and D. W. Dixon: Emerging strategies in microbial haem capture. *Mol Microbiol* 39 (1), 1 -11 (2001)
66. Grassme, H., V. Jendrosseck, and E. Gulbins: Molecular mechanisms of bacteria induced apoptosis. *Apoptosis* 6 (6), 441 -445 (2001)
67. Graves, D. T., M. Oskoui, S. Volejnikova, G. Naguib, S. Cai, T. Desta, A. Kakouras, and Y. Jiang: Tumor necrosis factor modulates fibroblast apoptosis, PMN recruitment, and osteoclast formation in response to *P. gingivalis* infection. *J Dent Res* 80 (10), 1875 -1879 (2001)
68. Grollman, A. P. and M. Moriya: Mutagenesis by 8-oxoguanine: an enemy within. *Trends Genet* 9 (7), 246 -249 (1993)
69. Gros, L., M. K. Saporbaev, and J. Laval: Enzymology of the repair of free radicals-induced DNA damage. *Oncogene* 21 (58), 8905 -8925 (2002)
70. Grossmann, J.: Molecular mechanisms of "detachment-induced apoptosis--Anoikis". *Apoptosis* 7 (3), 247 -260 (2002)
71. Gu, J. and N. Taniguchi: Regulation of integrin functions by N-glycans. *Glycoconj J* 21 (1-2), 9 -15 (2004)
72. Guimaraes, C. A. and R. Linden: Programmed cell deaths. Apoptosis and alternative deathstyles. *Eur J Biochem* 271 (9), 1638 -1650 (2004)
73. Guiney, D. G.: The role of host cell death in *Salmonella* infections. *Curr Top Microbiol Immunol* 289 131 -150 (2005)
74. Gwak, G. Y., J. H. Yoon, S. J. Yu, S. C. Park, J. J. Jang, K. B. Lee, S. H. Lee, S. M. Lee, C. S. Shin, K. S. Suh, and H. S. Lee: Anti-apoptotic N-cadherin signaling and its prognostic implication in human hepatocellular carcinomas. *Oncol Rep* 15 (5), 1117 -1123 (2006)
75. Handfield, M., J. J. Mans, G. Zheng, M. C. Lopez, S. Mao, A. Prohulske-Fox, G. Narasimhan, H. V. Baker, and R. J. Lamont: Distinct transcriptional profiles characterize oral epithelium-microbiota interactions. *Cell Microbiol* 7 (6), 811 -823 (2005)
76. Harris, J. I., R. R. B. Russell, M. A. Curtis, J. Aduse-Opoku, and J. J. Taylor: Molecular mediators of *Porphyromonas gingivalis*-induced T-cell apoptosis. *Oral Microbiol Immunol* 17 (4), 224 -230 (2002)
77. He, J., H. Miyazaki, C. Anaya, F. Yu, W. A. Yeudall, and J. P. Lewis: Role of *Porphyromonas gingivalis* FeoB2 in metal uptake and oxidative stress protection. *Infect Immun* 74 (7), 4214 -4223 (2006)

78. Henry, L., Johnson N A, and Fletcher H.M.: The role of *uvrB* in UV sensitivity and oxidative stress in *Porphyromonas gingivalis* W83. *Journal of Dental Research* 86 (Spec Iss A) Abstract# 1819 IADR 85th General Session, New Orleans, LA 86 (Special Issue A), (2007)
79. Hintermann, E., S. K. Haake, U. Christen, A. Sharabi, and V. Quaranta: Discrete proteolysis of focal contact and adherens junction components in *Porphyromonas gingivalis*-infected oral keratinocytes: a strategy for cell adhesion and migration disabling. *Infect Immun* 70 (10), 5846-5856 (2002)
80. Holt, S. C. and J. L. Ebersole: *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*: the "red complex", a prototype polybacterial pathogenic consortium in periodontitis. *Periodontol* 2000 38 72-122 (2005)
81. Holt, S. C., L. Kesavalu, S. Walker, and C. A. Genco: Virulence factors of *Porphyromonas gingivalis*. *Periodontol* 2000 20 168-238 (1999)
82. Hosogi, Y. and M. J. Duncan: Gene expression in *Porphyromonas gingivalis* after contact with human epithelial cells. *Infect Immun* 73 (4), 2327-2335 (2005)
83. Huang, G. T., D. Kim, J. K. Lee, H. K. Kuramitsu, and S. K. Haake: Interleukin-8 and intercellular adhesion molecule 1 regulation in oral epithelial cells by selected periodontal bacteria: multiple effects of *Porphyromonas gingivalis* via antagonistic mechanisms. *Infect Immun* 69 (3), 1364-1372 (2001)
84. Ilic, D., E. A. Almeida, D. D. Schlaepfer, P. Dazin, S. Aizawa, and C. H. Damsky: Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. *J Cell Biol* 143 (2), 547-560 (1998)
85. Imamura, T.: The role of gingipains in the pathogenesis of periodontal disease. *J Periodontol* 74 (1), 111-118 (2003)
86. Imlay, J. A.: How oxygen damages microbes: oxygen tolerance and obligate anaerobiosis. *Adv Microb Physiol* 46 111-153 (2002)
87. Imlay, J. A. and S. Linn: DNA damage and oxygen radical toxicity. *Science* 240 (4857), 1302-1309 (1988)
88. Inaba, H., S. Kawai, T. Kato, I. Nakagawa, and A. Amano: Association between epithelial cell death and invasion by microspheres conjugated to *Porphyromonas gingivalis* vesicles with different types of fimbriae. *Infect Immun* 74 (1), 734-739 (2006)
89. Inayat-Hussain, S. H. and D. Ross: Intrinsic pathway of hydroquinone induced apoptosis occurs via both caspase-dependent and caspase-independent mechanisms. *Chem Res Toxicol* 18 (3), 420-427 (2005)
90. Isogai, E., H. Isogai, K. Kimura, N. Fujii, S. Takagi, K. Hirose, and M. Hayashi: *In vivo* induction of apoptosis and immune responses in mice by administration of lipopolysaccharide from *Porphyromonas gingivalis*. *Infect Immun* 64 (4), 1461-1466 (1996)
91. Jaattela, M.: Programmed cell death: many ways for cells to die decently. *Ann Med* 34 (6), 480-488 (2002)
92. Jeong, W., M. K. Cha, and I. H. Kim: Thioredoxin-dependent hydroperoxide peroxidase activity of bacterioferritin comigratory protein (BCP) as a new member of the thiol-specific antioxidant protein (TSA)/Alkyl hydroperoxide peroxidase C (AhpC) family. *J Biol Chem* 275 (4), 2924-2930 (2000)
93. Johansson, A. and S. Kalfas: Characterization of the proteinase-dependent cytotoxicity of *Porphyromonas gingivalis*. *Eur J Oral Sci* 106 (4), 863-871 (1998)
94. Johnson, D. E.: Noncaspase proteases in apoptosis. *Leukemia* 14 (9), 1695-1703 (2000)
95. Johnson, N. A. and Fletcher H.M.: The *bcp* gene in the *bcp-recA-vimA* operon is important to oxidative stress resistance in *Porphyromonas gingivalis* W83. *American Society for Microbiology 104th General Meeting Abstract # I-023 New Orleans, LA* (2004)
96. Johnson, N. A., Y. Liu, and H. M. Fletcher: Alkyl hydroperoxide peroxidase subunit C (*ahpC*) protects against organic peroxides but does not affect the virulence of *Porphyromonas gingivalis* W83. *Oral Microbiol Immunol* 19 (4), 233-239 (2004)
97. Johnson, N. A., R. McKenzie, L. McLean, L. C. Sowers, and H. M. Fletcher: 8-Oxo-7,8-dihydroguanine is removed by a nucleotide excision repair-like mechanism in *Porphyromonas gingivalis* W83. *J Bacteriol* 186 (22), 7697-7703 (2004)
98. Katz, J., V. Sambandam, J. H. Wu, S. M. Michalek, and D. F. Balkovetz: Characterization of *Porphyromonas gingivalis*-induced degradation of epithelial cell junctional complexes. *Infect Immun* 68 (3), 1441-1449 (2000)
99. Katz, J., Q. B. Yang, P. Zhang, J. Potempa, J. Travis, S. M. Michalek, and D. F. Balkovetz: Hydrolysis of epithelial junctional proteins by *Porphyromonas gingivalis* gingipains. *Infection and Immunity* 70 (5), 2512-2518 (2002)
100. Khlgatian, M., H. Nassar, H. H. Chou, F. C. Gibson, III, and C. A. Genco: Fimbria-dependent activation of cell adhesion molecule expression in *Porphyromonas gingivalis*-infected endothelial cells. *Infect Immun* 70 (1), 257-267 (2002)
101. Kikuchi, Y., N. Ohara, K. Sato, M. Yoshimura, H. Yukitake, E. Sakai, M. Shoji, M. Naito, and K. Nakayama: Novel stationary-phase-upregulated protein of *Porphyromonas gingivalis* influences production of superoxide dismutase, thiol peroxidase and thioredoxin. *Microbiology* 151 (Pt 3), 841-853 (2005)
102. Kim, D. Y. and K. K. Kim: Crystallization and preliminary X-ray studies of the protease domain of the heat-shock protein HtrA from *Thermotoga maritima*. *Acta Crystallogr D Biol Crystallogr* 58 (Pt 1), 170-172 (2002)
103. Kim, J. and S. Amar: Periodontal disease and systemic conditions: a bidirectional relationship. *Odontology* 94 (1), 10-21 (2006)
104. Konkel, M. E. and K. Tilly: Temperature-regulated expression of bacterial virulence genes. *Microbe Infect* 2 (2), 157-166 (2000)
105. Konola, J. T., K. E. Sargent, and J. B. Gow: Efficient repair of hydrogen peroxide-induced DNA damage by *Escherichia coli* requires SOS induction of RecA and RuvA proteins. *Mutat Res* 459 (3), 187-194 (2000)
106. Krokkan, H. E., R. Standal, and G. Slupphaug: DNA glycosylases in the base excision repair of DNA. *Biochem J* 325 (Pt 1) 1-16 (1997)
107. Kuboniwa, M., A. Amano, and S. Shizukuishi: Hemoglobin-binding protein purified from *Porphyromonas gingivalis* is identical to lysine-specific cysteine proteinase

- (Lys-Gingipain). *Biochem Biophys Res Commun* 249 (1), 38-43 (1998)
108. Kurita-Ochiai, T., K. Ochiai, and K. Fukushima: Volatile fatty acid, metabolic by-product of periodontopathic bacteria, induces apoptosis in WEHI 231 and RAJI B lymphoma cells and splenic B cells. *Infect Immun* 66 (6), 2587-2594 (1998)
 109. Kurita-Ochiai, T., K. Ochiai, and K. Fukushima: Butyric acid-induced T-cell apoptosis is mediated by caspase-8 and -9 activation in a Fas-independent manner. *Clin Diagn Lab Immunol* 8 (2), 325-332 (2001)
 110. Lee, S. D., W. W. Kuo, D. Y. Lin, T. H. Chen, W. H. Kuo, H. H. Hsu, J. Z. Chen, J. Y. Liu, Y. L. Yeh, and C. Y. Huang: Role of calcineurin in Porphyromonas gingivalis-induced myocardial cell hypertrophy and apoptosis. *J Biomed Sci* 1-10 (2005)
 111. Lee, S. D., C. C. Wu, W. W. Kuo, J. A. Lin, J. M. Hwang, M. C. Lu, L. M. Chen, H. H. Hsu, C. K. Wang, S. H. Chang, and C. Y. Huang: Porphyromonas gingivalis-related cardiac cell apoptosis was majorly co-activated by p38 and extracellular signal-regulated kinase pathways. *J Periodontal Res* 41 (1), 39-46 (2006)
 112. Lee, S. G., V. Pancholi, and V. A. Fischetti: Characterization of a unique glycosylated anchor endopeptidase that cleaves the LPXTG sequence motif of cell surface proteins of Gram-positive bacteria. *J Biol Chem* 277 (49), 46912-46922 (2002)
 113. Leist, M. and M. Jaattela: Four deaths and a funeral: from caspases to alternative mechanisms. *Nat Rev Mol Cell Biol* 2 (8), 589-598 (2001)
 114. Lewis, J. P., J. Dawson, K. Jones, J. Hannis, D. Muddiman, H. Birkedal-Hansen, and F. L. Macrina: Hemoglobinase activity of the lysine protease of Porphyromonas gingivalis. *J Dent Res* 78 506- (1999)
 115. Lewis, J. P., J. A. Dawson, J. C. Hannis, D. Muddiman, and F. L. Macrina: Hemoglobinase activity of the lysine gingipain protease (Kgp) of Porphyromonas gingivalis W83. *J Bacteriol* 181 (16), 4905-4913 (1999)
 116. Lipinska, B., M. Zylicz, and C. Georgopoulos: The HtrA (DegP) protein, essential for Escherichia coli survival at high temperatures, is an endopeptidase. *J Bacteriol* 172 (4), 1791-1797 (1990)
 117. Liu, Y. and H. M. Fletcher: Environmental regulation of recA gene expression in Porphyromonas gingivalis. *Oral Microbiol Immunol* 16 (3), 136-143 (2001)
 118. Liu, Y. and H. M. Fletcher: The recA gene in Porphyromonas gingivalis is expressed during infection of the murine host. *Oral Microbiol Immunol* 16 (4), 218-223 (2001)
 119. Lu, A. L., X. Li, Y. Gu, P. M. Wright, and D. Y. Chang: Repair of oxidative DNA damage: mechanisms and functions. *Cell Biochem Biophys* 35 (2), 141-170 (2001)
 120. Luder, C. G. and U. Gross: Apoptosis and its modulation during infection with Toxoplasma gondii: molecular mechanisms and role in pathogenesis. *Curr Top Microbiol Immunol* 289 219-237 (2005)
 121. Lynch, M. C. and H. K. Kuramitsu: Role of superoxide dismutase activity in the physiology of Porphyromonas gingivalis. *Infect Immun* 67 (7), 3367-3375 (1999)
 122. Mao, S., Y. Park, Y. Hasegawa, G. D. Tribble, C. E. James, M. Handfield, M. F. Stavropoulos, O. Yilmaz, and R. J. Lamont: Intrinsic apoptotic pathways of gingival epithelial cells modulated by Porphyromonas gingivalis. *Cell Microbiol* (2007)
 123. Marnett, L. J.: Oxy radicals, lipid peroxidation and DNA damage. *Toxicology* 181-182 219-222 (2002)
 124. Mathiasen, I. S. and M. Jaattela: Triggering caspase-independent cell death to combat cancer. *Trends Mol Med* 8 (5), 212-220 (2002)
 125. McKenzie, R. and Fletcher H.M.: Studies in oxidative stress resistance in Porphyromonas gingivalis. *Journal of Dental Research* 86 (Spec Iss A) Abstract# 2353 IADR 85th General Session New Orleans, LA (2007)
 126. Mekalanos, J. J.: Environmental signals controlling expression of virulence determinants in bacteria. *J Bacteriol* 174 1-7 (1992)
 127. Meredith, J. E., Jr., B. Fazeli, and M. A. Schwartz: The extracellular matrix as a cell survival factor. *Mol Biol Cell* 4 (9), 953-961 (1993)
 128. Mezyk-Kopec, R., M. Bzowska, J. Potempa, M. Bzowska, N. Jura, A. Sroka, R. A. Black, and J. Bereta: Inactivation of membrane tumor necrosis factor alpha by gingipains from Porphyromonas gingivalis. *Infect Immun* 73 (3), 1506-1514 (2005)
 129. Michalek, S. M., J. Katz, N. K. Childers, M. Martin, and D. F. Balkovetz: Microbial/host interactions: mechanisms involved in host responses to microbial antigens. *Immunol Res* 26 (1-3), 223-234 (2002)
 130. Michel, J. B.: Anoikis in the Cardiovascular System. Known and Unknown Extracellular Mediators. *Arterioscler Thromb Vasc Biol* (2003)
 131. Mikolajczyk, J., K. M. Boatright, H. R. Stennicke, T. Nazif, J. Potempa, M. Bogoy, and G. S. Salvesen: Sequential autolytic processing activates the zymogen of Arg-gingipain. *J Biol Chem* 278 (12), 10458-10464 (2003)
 132. Mikolajczyk-Pawlinska, J., T. Kordula, N. Pavloff, P. A. Pemberton, W. C. Chen, J. Travis, and J. Potempa: Genetic variation of Porphyromonas gingivalis genes encoding gingipains, cysteine proteinases with arginine or lysine specificity. *Biol Chem Hoppe Seyler* 379 (2), 205-211 (1998)
 133. Miller, R. A. and B. E. Britigan: Role of oxidants in microbial pathophysiology. *Clin Microbiol Rev* 10 (1), 1-18 (1997)
 134. Moens, S. and J. Vanderleyden: Glycoproteins in prokaryotes. *Arch Microbiol* 168 (3), 169-175 (1997)
 135. Monack, D. M., A. Mueller, and S. Falkow: Persistent bacterial infections: the interface of the pathogen and the host immune system. *Nat Rev Microbiol* 2 (9), 747-765 (2004)
 136. Monteiro, H. P., E. F. Silva, and A. Stern: Nitric oxide: a potential inducer of adhesion-related apoptosis--anoikis. *Nitric Oxide* 10 (1), 1-10 (2004)
 137. Morioka, M., D. Hinode, A. Nagata, H. Hayashi, S. Ichimiya, M. Ueda, R. Kido, and R. Nakamura: Cytotoxicity of Porphyromonas gingivalis toward cultured human gingival fibroblasts. *Oral Microbiol Immunol* 8 (4), 203-207 (1993)

138. Moss, J. E., A. O. Aliprantis, and A. Zychlinsky: The regulation of apoptosis by microbial pathogens. *Int Rev Cytol* 187 203 -259 (1999)
139. Mu, D., M. Tursun, D. R. Duckett, J. T. Drummond, P. Modrich, and A. Sancar: Recognition and repair of compound DNA lesions (base damage and mismatch) by human mismatch repair and excision repair systems. *Mol Cell Biol* 17 (2), 760 -769 (1997)
140. Murray, D. A. and J. M. Wilton: Lipopolysaccharide from the periodontal pathogen *Porphyromonas gingivalis* prevents apoptosis of HL60-derived neutrophils *in vitro*. *Infect Immun* 71 (12), 7232 -7235 (2003)
141. Nakayama, K.: Domain-specific rearrangement between the two Arg-gingipain- encoding genes in *Porphyromonas gingivalis*: possible involvement of nonreciprocal recombination. *Microbiol Immunol* 41 (3), 185 -196 (1997)
142. Nakayama, K.: Molecular genetics of *Porphyromonas gingivalis*: gingipains and other virulence factors. *Curr Protein Pept Sci* 4 (6), 389 -395 (2003)
143. Nakayama, K., F. Yoshimura, T. Kadowaki, and K. Yamamoto: Involvement of arginine-specific cysteine proteinase (Arg-gingipain) in fimbriation of *Porphyromonas gingivalis*. *J Bacteriol* 178 (10), 2818 - 2824 (1996)
144. Nakhjiri, S. F., Y. Park, O. Yilmaz, W. O. Chung, K. Watanabe, A. El Sabaeny, K. Park, and R. J. Lamont: Inhibition of epithelial cell apoptosis by *Porphyromonas gingivalis*. *FEMS Microbiol Lett* 200 (2), 145 -149 (2001)
145. Nelson, K. E., R. D. Fleischmann, R. T. DeBoy, I. T. Paulsen, D. E. Fouts, J. A. Eisen, S. C. Daugherty, R. J. Dodson, A. S. Durkin, M. Gwinn, D. H. Haft, J. F. Kolonay, W. C. Nelson, T. Mason, L. Tallon, J. Gray, D. Granger, H. Tettelin, H. Dong, J. L. Galvin, M. J. Duncan, F. E. Dewhirst, and C. M. Fraser: Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83. *J Bacteriol* 185 (18), 5591 -5601 (2003)
146. Nguyen, K. A., J. Travis, and J. Potempa: Does the importance of the C-terminal residues in the maturation of RgpB from *Porphyromonas gingivalis* reveal a novel mechanism for protein export in a subgroup of Gram-Negative bacteria? *J Bacteriol* 189 (3), 833 -843 (2007)
147. Ohara, N., Y. Kikuchi, M. Shoji, M. Naito, and K. Nakayama: Superoxide dismutase-encoding gene of the obligate anaerobe *Porphyromonas gingivalis* is regulated by the redox-sensing transcription activator OxyR. *Microbiology* 152 (Pt 4), 955 -966 (2006)
148. Okamoto, K., K. Nakayama, T. Kadowaki, N. Abe, D. B. Ratnayake, and K. Yamamoto: Involvement of a lysine-specific cysteine proteinase in hemoglobin adsorption and heme accumulation by *Porphyromonas gingivalis*. *J Biol Chem* 273 (33), 21225 -21231 (1998)
149. Okano, S., Y. Shibata, T. Shiroza, and Y. Abiko: Proteomics-based analysis of a counter-oxidative stress system in *Porphyromonas gingivalis*. *Proteomics* 6 (1), 251 -258 (2006)
150. Olango, G. J., F. Roy, S. M. Sheets, M. K. Young, and H. M. Fletcher: Gingipain RgpB is excreted as a proenzyme in the *vimA*-defective mutant *Porphyromonas gingivalis* FLL92. *Infect Immun* 71 (7), 3740 -3747 (2003)
151. Olczak, T., D. W. Dixon, and C. A. Genco: Binding specificity of the *Porphyromonas gingivalis* heme and hemoglobin receptor HmuR, gingipain K, and gingipain R1 for heme, porphyrins, and metalloporphyrins. *J Bacteriol* 183 (19), 5599 -5608 (2001)
152. Olczak, T., W. Simpson, X. Liu, and C. A. Genco: Iron and heme utilization in *Porphyromonas gingivalis*. *FEMS Microbiol Rev* 29 (1), 119 -144 (2005)
153. Ozaki, K. and S. Hanazawa: *Porphyromonas gingivalis* fimbriae inhibit caspase-3-mediated apoptosis of monocytic THP-1 cells under growth factor deprivation via extracellular signal-regulated kinase-dependent expression of p21 Cip/WAF1. *Infect Immun* 69 (8), 4944 -4950 (2001)
154. Pacheco, F. J., J. Servin, D. Dang, J. Kim, C. Molinaro, T. Daniels, T. A. Brown-Bryan, M. Imoto-Egami, and C. A. Casiano: Involvement of lysosomal cathepsins in the cleavage of DNA topoisomerase I during necrotic cell death. *Arthritis Rheum* 52 (7), 2133 -2145 (2005)
155. Pallen, M. J. and B. W. Wren: The HtrA family of serine proteases. *Mol Microbiol* 26 (2), 209 -221 (1997)
156. Park, Y. and R. J. Lamont: Contact-dependent protein secretion in *Porphyromonas gingivalis*. *Infection and Immunity* 66 (10), 4777 -4782 (1998)
157. Pavloff, N., P. A. Pemberton, J. Potempa, W. C. A. Chen, R. N. Pike, V. Prochazka, M. C. Kiefer, J. Travis, and P. J. Barr: Molecular cloning and characterization of *Porphyromonas gingivalis* lysine-specific gingipain - A new member of an emerging family of pathogenic bacterial cysteine proteinases. *J Biol Chem* 272 (3), 1595 -1600 (1997)
158. Pavloff, N., J. Potempa, R. N. Pike, V. Prochazka, M. C. Kiefer, J. Travis, and P. J. Barr: Molecular cloning and structural characterization of the Arg-gingipain proteinase of *Porphyromonas gingivalis*. Biosynthesis as a proteinase-adhesin polyprotein. *J Biol Chem* 270 1007 - 1010 (1995)
159. Percival, R. S., P. D. Marsh, D. A. Devine, M. Rangarajan, J. Aduse-Opoku, P. Shepherd, and M. A. Curtis: Effect of temperature on growth, hemagglutination, and protease activity of *Porphyromonas gingivalis*. *Infect Immun* 67 (4), 1917 -1921 (1999)
160. Poquet, I., V. Saint, E. Seznec, N. Simoes, A. Bolotin, and A. Gruss: HtrA is the unique surface housekeeping protease in *Lactococcus lactis* and is required for natural protein processing. *Mol Microbiol* 35 (5), 1042 -1051 (2000)
161. Potempa, J., A. Banbula, and J. Travis: Role of bacterial proteinases in matrix destruction and modulation of host responses. *Periodontol* 2000 12 153 -192 (2000)
162. Potempa, J., N. Pavloff, and J. Travis: *Porphyromonas gingivalis*: a proteinase/gene accounting audit. *Trends Microbiol* 3 (11), 430 -434 (1995)
163. Potempa, J., A. Sroka, T. Imamura, and J. Travis: Gingipains, the major cysteine proteinases and virulence factors of *Porphyromonas gingivalis*: structure, function and assembly of multidomain protein complexes. *Curr Protein Pept Sci* 4 (6), 397 -407 (2003)
164. Price, D. T. and J. Loscalzo: Cellular adhesion molecules and atherogenesis. *Am J Med* 107 (1), 85 -97 (1999)

165. Rangarajan, M., A. Hashim, J. duse-Opoku, N. Paramonov, E. F. Hounsell, and M. A. Curtis: Expression of Arg-Gingipain RgpB is required for correct glycosylation and stability of monomeric Arg-gingipain RgpA from *Porphyromonas gingivalis* W50. *Infect Immun* 73 (8), 4864 -4878 (2005)
166. Re, F., A. Zanetti, M. Sironi, N. Polentarutti, L. Lanfranccone, E. Dejana, and F. Colotta: Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J Cell Biol* 127 (2), 537 - 546 (1994)
167. Reddig, P. J. and R. L. Juliano: Clinging to life: cell to matrix adhesion and cell survival. *Cancer Metastasis Rev* 24 (3), 425 -439 (2005)
168. Robles-Price, A., Reid K, and Fletcher H.M.: The role of MutY in *Porphyromonas gingivalis* W83. *Journal of Dental Research* 86 (Spec Iss A) Abstract# 2354 IADR 85th General Session New Orleans, LA 86 (2007)
169. Roy, F., E. Vanterpool, and H. M. Fletcher: HtrA in *Porphyromonas gingivalis* can regulate growth and gingipain activity under stressful environmental conditions. *Microbiology* 152 (Pt 11), 3391 -3398 (2006)
170. Rytomaa, M., L. M. Martins, and J. Downward: Involvement of FADD and caspase-8 signalling in detachment-induced apoptosis. *Curr Biol* 9 (18), 1043 - 1046 (1999)
171. Salyers, A. A.: Bacterial pathogenesis: a molecular approach. 1 -418 (1994)
172. Sancar, A. and W. D. Rupp: A novel repair enzyme: UVRABC excision nuclease of *Escherichia coli* cuts a DNA strand on both sides of the damaged region. *Cell* 33 (1), 249 -260 (1983)
173. Sawamoto, Y., N. Sugano, H. Tanaka, and K. Ito: Detection of periodontopathic bacteria and an oxidative stress marker in saliva from periodontitis patients. *Oral Microbiol Immunol* 20 (4), 216 -220 (2005)
174. Scott, A. D., M. Neishabury, D. H. Jones, S. H. Reed, S. Boiteux, and R. Waters: Spontaneous mutation, oxidative DNA damage, and the roles of base and nucleotide excision repair in the yeast *Saccharomyces cerevisiae*. *Yeast* 15 (3), 205 -218 (1999)
175. Scragg, M. A., A. Alsam, M. Rangarajan, J. M. Slaney, P. Shepherd, D. M. Williams, and M. A. Curtis: Nuclear targeting of *Porphyromonas gingivalis* W50 protease in epithelial cells. *Infect Immun* 70 (10), 5740 - 5750 (2002)
176. Scragg, M. A., S. J. Cannon, M. Rangarajan, D. M. Williams, and M. A. Curtis: Targeted disruption of fibronectin-integrin interactions in human gingival fibroblasts by the RI protease of *Porphyromonas gingivalis* W50. *Infect Immun* 67 (4), 1837 -1843 (1999)
177. Seaver, L. C. and J. A. Imlay: Alkyl hydroperoxide reductase is the primary scavenger of endogenous hydrogen peroxide in *Escherichia coli*. *J Bacteriol* 183 (24), 7173 - 7181 (2001)
178. Seers, C. A., N. Slakeski, P. D. Veith, T. Nikolof, Y. Y. Chen, S. G. Dashper, and E. C. Reynolds: The RgpB C-terminal domain has a role in attachment of RgpB to the outer membrane and belongs to a novel C-terminal-domain family found in *Porphyromonas gingivalis*. *J Bacteriol* 188 (17), 6376 -6386 (2006)
179. Sekiguchi, M. and T. Tsuzuki: Oxidative nucleotide damage: consequences and prevention. *Oncogene* 21 (58), 8895 -8904 (2002)
180. SETLOW, R. B. and W. L. CARRIER: The disappearance of thymine dimers from DNA: An error correcting mechanism. *Proc Natl Acad Sci U S A* 51 226 - 231 (1964)
181. Shah, H. N., S. E. Gharbia, and C. M. O'Toole: Assessment of the relative cytotoxicity of *Porphyromonas gingivalis* cells, products, and components on human epithelial cell lines. *J Periodontol* 63 (1), 44 -51 (1992)
182. Sheets, S. M., J. Potempa, J. Travis, C. A. Casiano, and H. M. Fletcher: Gingipains from *Porphyromonas gingivalis* W83 induce cell adhesion molecule cleavage and apoptosis in endothelial cells. *Infect Immun* 73 (3), 1543 - 1552 (2005)
183. Sheets, S. M., J. Potempa, J. Travis, H. M. Fletcher, and C. A. Casiano: Gingipains from *Porphyromonas gingivalis* W83 synergistically disrupt endothelial cell adhesion and can induces caspase-independent apoptosis. *Infect Immun* In Press (2006)
184. Shelburne, C. E., R. M. Gleason, W. A. Coulter, M. S. Lantz, and D. E. Lopatin: Differential display analysis of *Porphyromonas gingivalis* gene activation response to heat and oxidative stress. *Oral Microbiol Immunol* 20 (4), 233 - 238 (2005)
185. Sherman, M. P. and K. Petrak: Lactoferrin-enhanced anoikis: a defense against neonatal necrotizing enterocolitis. *Med Hypotheses* 65 (3), 478 -482 (2005)
186. Shi, Y., D. B. Ratnayake, K. Okamoto, N. Abe, K. Yamamoto, and K. Nakayama: Genetic analyses of proteolysis, hemoglobin finding, and hemagglutination of *Porphyromonas gingivalis* - Construction of mutants with a combination of *rgpA*, *rgpB*, *kgp*, and *hagA*. *J Biol Chem* 274 (25), 17955 -17960 (1999)
187. Shirin, H., E. M. Sordillo, T. K. Kolevska, H. Hibshoosh, Y. Kawabata, S. H. Oh, J. F. Kuebler, T. Delohery, C. M. Weghorst, I. B. Weinstein, and S. F. Moss: Chronic *Helicobacter pylori* infection induces an apoptosis-resistant phenotype associated with decreased expression of p27 (kip1). *Infect Immun* 68 (9), 5321 -5328 (2000)
188. Shoji, M., D. B. Ratnayake, Y. Shi, T. Kadowaki, K. Yamamoto, F. Yoshimura, A. Akamine, M. A. Curtis, and K. Nakayama: Construction and characterization of a nonpigmented mutant of *Porphyromonas gingivalis*: cell surface polysaccharide as an anchorage for gingipains. *Microbiology* 148 (Pt 4), 1183 -1191 (2002)
189. Silveira, J. R., M. Kalafatis, and P. B. Tracy: Carbohydrate moieties on the procofactor factor V, but not the derived cofactor factor Va, regulate its inactivation by activated protein C. *Biochemistry* 41 (5), 1672 -1680 (2002)
190. Smalley, J. W. and A. J. Birss: Iron protoporphyrin IX albumin complexing increases the capacity and avidity of its binding to the periodontopathogen *Porphyromonas gingivalis*. *Microb Pathog* 26 (3), 131 -137 (1999)
191. Smalley, J. W., A. J. Birss, and J. Silver: The periodontal pathogen *Porphyromonas gingivalis* harnesses the chemistry of the m-oxo bishaem of iron protoporphyrin IX to protect against hydrogen peroxide. *FEMS Microbiol Lett* 183 (1), 159 -164 (2000)

192. Smalley, J. W., A. J. Birss, B. Szmigielski, and J. Potempa: The HA2 haemagglutinin domain of the lysine-specific gingipain (Kgp) of *Porphyromonas gingivalis* promotes micro-oxo bishaem formation from monomeric iron (III) protoporphyrin IX. *Microbiology* 152 (Pt 6), 1839 -1845 (2006)
193. Smalley, J. W., J. Silver, P. J. Marsh, and A. J. Birss: The periodontopathogen *Porphyromonas gingivalis* binds iron protoporphyrin IX in the m-oxo dimeric form: an oxidative buffer and possible pathogenic mechanism. *Biochem J* 331 (3), 681 -685 (1998)
194. Sreedhar, A. S. and P. Csermely: Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review. *Pharmacol Ther* 101 (3), 227 -257 (2004)
195. Storz, G. and J. A. Imlay: Oxidative stress. *Curr Opin Microbiol* 2 (2), 188 -194 (1999)
196. Stupack, D. G.: Integrins as a distinct subtype of dependence receptors. *Cell Death Differ* 12 (8), 1021 -1030 (2005)
197. Sugawara, S., E. Nemoto, H. Tada, K. Miyake, T. Imamura, and H. Takada: Proteolysis of human monocyte CD14 by cysteine proteinases (gingipains) from *Porphyromonas gingivalis* leading to lipopolysaccharide hyporesponsiveness. *J Immunol* 165 (1), 411 -418 (2000)
198. Sztukowska, M., M. Bugno, J. Potempa, J. Travis, and D. M. Kurtz, Jr.: Role of rubrerythrin in the oxidative stress response of *Porphyromonas gingivalis*. *Mol Microbiol* 44 (2), 479 -488 (2002)
199. Sztukowska, M., A. Sroka, M. Bugno, A. Banbula, Y. Takahashi, R. N. Pike, C. A. Genco, J. Travis, and J. Potempa: The C-terminal domains of the gingipain K polypeptide are necessary for assembly of the active enzyme and expression of associated activities. *Mol Microbiol* 54 (5), 1393 -1408 (2004)
200. Szymanski, C. M., D. H. Burr, and P. Guerry: *Campylobacter* protein glycosylation affects host cell interactions. *Infect Immun* 70 (4), 2242 -2244 (2002)
201. Szymanski, C. M. and B. W. Wren: Protein glycosylation in bacterial mucosal pathogens. *Nat Rev Microbiol* 3 (3), 225 -237 (2005)
202. Tada, H., S. Sugawara, E. Nemoto, T. Imamura, J. Potempa, J. Travis, H. Shimauchi, and H. Takada: Proteolysis of icam-1 on human oral epithelial cells by gingipains. *J Dent Res* 82 (10), 796 -801 (2003)
203. Takeuchi, T., N. Kato, K. Watanabe, and K. Morimoto: Mechanism of oxidative DNA damage induction in a strict anaerobe, *Prevotella melaninogenica*. *FEMS Microbiol Lett* 192 (1), 133 -138 (2000)
204. Tanjoni, I., R. Weinlich, M. S. Della-Casa, P. B. Clissa, R. F. Saldanha-Gama, M. S. de Freitas, C. Barja-Fidalgo, G. P. Amarante-Mendes, and A. M. Moura-da-Silva: Jararhagin, a snake venom metalloproteinase, induces a specialized form of apoptosis (anoikis) selective to endothelial cells. *Apoptosis* 10 (4), 851 -861 (2005)
205. Tchou, J. and A. P. Grollman: Repair of DNA containing the oxidatively-damaged base, 8-oxoguanine. *Mutat Res* 299 (3-4), 277 -287 (1993)
206. Theis, K., M. Skovvaga, M. Machius, N. Nakagawa, B. Van Houten, and C. Kisker: The nucleotide excision repair protein UvrB, a helicase-like enzyme with a catch. *Mutat Res* 460 (3-4), 277 -300 (2000)
207. Thornberry, N. A. and Y. Lazebnik: Caspases: enemies within. *Science* 281 (5381), 1312 -1316 (1998)
208. Turk, B., V. Stoka, J. Rozman-Pungercar, T. Cirman, G. Droga-Mazovec, K. Oresic, and V. Turk: Apoptotic pathways: involvement of lysosomal proteases. *Biol Chem* 383 (7-8), 1035 -1044 (2002)
209. Ueshima, J., M. Shoji, D. B. Ratnayake, K. Abe, S. Yoshida, K. Yamamoto, and K. Nakayama: Purification, gene cloning, gene expression, and mutants of Dps from the obligate anaerobe *Porphyromonas gingivalis*. *Infect Immun* 71 (3), 1170 -1178 (2003)
210. Upreti, R. K., M. Kumar, and V. Shankar: Bacterial glycoproteins: functions, biosynthesis and applications. *Proteomics* 3 (4), 363 -379 (2003)
211. Urnowey, S., T. Ansai, V. Bitko, K. Nakayama, T. Takehara, and S. Barik: Temporal activation of anti- and pro-apoptotic factors in human gingival fibroblasts infected with the periodontal pathogen, *Porphyromonas gingivalis*: potential role of bacterial proteases in host signalling. *BMC Microbiol* 6 26 - (2006)
212. US Department of Health and Human Services: Oral Health in America: A Report of the Surgeon General. (2000)
213. Vanterpool, E., F. Roy, and H. M. Fletcher: The *vimE* gene downstream of *vimA* is independently expressed and is involved in modulating proteolytic activity in *Porphyromonas gingivalis* W83. *Infect Immun* 72 (10), 5555 -5564 (2004)
214. Vanterpool, E., F. Roy, and H. M. Fletcher: Inactivation of *vimF*, a Putative Glycosyltransferase Gene Downstream of *vimE*, Alters Glycosylation and Activation of the Gingipains in *Porphyromonas gingivalis* W83. *Infect Immun* 73 (7), 3971 -3982 (2005)
215. Vanterpool, E., F. Roy, L. Sandberg, and H. M. Fletcher: Altered gingipain maturation in *vimA*- and *vimE*-defective isogenic mutants of *Porphyromonas gingivalis*. *Infect Immun* 73 (3), 1357 -1366 (2005)
216. Vanterpool, E., F. Roy, W. Zhan, S. M. Sheets, L. Sangberg, and H. M. Fletcher: *VimA* is part of the maturation pathway for the major gingipains of *Porphyromonas gingivalis* W83. *Microbiology* 152 (Pt 11), 3383 -3389 (2006)
217. Varki, A.: Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* 3 (2), 97 -130 (1993)
218. Vaux, D. L. and A. Strasser: The molecular biology of apoptosis. *Proc Natl Acad Sci U S A* 93 (6), 2239 -2244 (1996)
219. Veith, P. D., Y. Y. Chen, and E. C. Reynolds: *Porphyromonas gingivalis* RgpA and Kgp proteinases and adhesins are C terminally processed by the carboxypeptidase CPG70. *Infect Immun* 72 (6), 3655 -3657 (2004)
220. Walter, C., J. Zahlten, B. Schmeck, C. Schaudinn, S. Hippenstiel, E. Frisch, A. C. Hocke, N. Pischon, H. K. Kuramitsu, J. P. Bernimoulin, N. Suttorp, and M. Krull: *Porphyromonas gingivalis* strain-dependent activation of human endothelial cells. *Infect Immun* 72 (10), 5910 -5918 (2004)
221. Wandersman, C.: Secretion, processing and activation of bacterial extracellular proteases. *Mol Microbiol* 3 (12), 1825 -1831 (1989)
222. Wang, P. L., M. Shinohara, N. Murakawa, M. Endo, S. Sakata, M. Okamura, and K. Ohura: Effect of cysteine

protease of *Porphyromonas gingivalis* on adhesion molecules in gingival epithelial cells. *Jpn J Pharmacol* 80 (1), 75 -79 (1999)

223. Wang, P. L., S. Shirasu, M. Shinohara, M. Daito, M. Oido, Y. Kowashi, and K. Ohura: Induction of apoptosis in human gingival fibroblasts by a *Porphyromonas gingivalis* protease preparation. *Arch Oral Biol* 44 (4), 337 -342 (1999)

224. Watanabe, K., O. Yilmaz, S. F. Nakhjiri, C. M. Belton, and R. J. Lamont: Association of mitogen-activated protein kinase pathways with gingival epithelial cell responses to *Porphyromonas gingivalis* infection. *Infect Immun* 69 (11), 6731 -6737 (2001)

225. Waterhouse, N. J., K. A. Sedelies, V. R. Sutton, M. J. Pinkoski, K. Y. Thia, R. Johnstone, P. I. Bird, D. R. Green, and J. A. Trapani: Functional dissociation of DeltaPsim and cytochrome c release defines the contribution of mitochondria upstream of caspase activation during granzyme B-induced apoptosis. *Cell Death Differ* 13 (4), 607 -618 (2006)

226. Wei, L., Y. Yang, X. Zhang, and Q. Yu: Cleavage of p130Cas in anoikis. *J Cell Biochem* 91 (2), 325 -335 (2004)

227. Weinrauch, Y. and A. Zychlinsky: The induction of apoptosis by bacterial pathogens. *Annu Rev Microbiol* 53 155 -187 (1999)

228. Wilson, C. A. and J. L. Browning: Death of HT29 adenocarcinoma cells induced by TNF family receptor activation is caspase-independent and displays features of both apoptosis and necrosis. *Cell Death Differ* 9 (12), 1321 -1333 (2002)

229. Xie, H., W. O. Chung, Y. Park, and R. J. Lamont: Regulation of the *Porphyromonas gingivalis* *fimA* (fimbriillin) gene. *Infection and Immunity* 68 (12), 6574 -6579 (2000)

230. Yilmaz, O., T. Jungas, P. Verbeke, and D. M. Ojcius: Activation of the phosphatidylinositol 3-kinase/Akt pathway contributes to survival of primary epithelial cells infected with the periodontal pathogen *Porphyromonas gingivalis*. *Infect Immun* 72 (7), 3743 -3751 (2004)

231. Yilmaz, O., P. Verbeke, R. J. Lamont, and D. M. Ojcius: Intercellular spreading of *Porphyromonas gingivalis* infection in primary gingival epithelial cells. *Infect Immun* 74 (1), 703 -710 (2006)

232. Yun, P. L., A. A. Decarlo, and N. Hunter: Gingipains of *Porphyromonas gingivalis* modulate leukocyte adhesion molecule expression induced in human endothelial cells by ligation of CD99. *Infect Immun* 74 (3), 1661 -1672 (2006)

233. Zhan, M., H. Zhao, and Z. C. Han: Signalling mechanisms of anoikis. *Histol Histopathol* 19 (3), 973 -983 (2004)

234. Zhang, D., P. J. Beresford, A. H. Greenberg, and J. Lieberman: Granzymes A and B directly cleave lamins and disrupt the nuclear lamina during granule-mediated cytotoxicity. *Proc Natl Acad Sci U S A* 98 (10), 5746 -5751 (2001)

235. Zhang, D., M. S. Pasternack, P. J. Beresford, L. Wagner, A. H. Greenberg, and J. Lieberman: Induction of rapid histone degradation by the cytotoxic T lymphocyte protease Granzyme A. *J Biol Chem* 276 (5), 3683 -3690 (2001)

236. Zhang, W., M. Shokeen, D. Li, and J. L. Mehta: Identification of apoptosis-inducing factor in human coronary artery endothelial cells. *Biochem Biophys Res Commun* 301 (1), 147 -151 (2003)

237. Zhou, Q. and S. Amar: Identification of proteins differentially expressed in human monocytes exposed to *Porphyromonas gingivalis* and its purified components by high-throughput immunoblotting. *Infect Immun* 74 (2), 1204 -1214 (2006)

238. Ziegler, U. and P. Groscurth: Morphological features of cell death. *News Physiol Sci* 19 124 -128 (2004)

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