GINGIPAIN

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The gram-negative, anaerobic bacterium Porphyromonas gingivalis is associated with adult periodontal disease in humans and uses a number of adhesin molecules, including fimbriae, hemagglutinins and protease/adhesion complexes, to aid colonization of the oral cavity. Although each of these molecules possesses a degree of functional redundancy with respect to one another, it is becoming apparent that each molecule may in fact have a preferred, discrete role under the competitive conditions found within the dynamic environment of the oral cavity in vivo. Porphyromonas gingivalis, employs a number of pathogenic mechanisms, including protease/adhesin complexes (gingipains), fimbriae and hemagglutinins, to maintain attachment within colonized hosts. The gingipain protease/adhesion complexes are believed to allow bacterial cells to associate intimately with proteins in the gum matrix, prior to the secretion and dissemination of the enzymes through the oral cavity leading to the destruction of integral matrix proteins. It is likely that the gingipains are dominant factors in the physically destructive aspects of infection and associated diseases. This review article reviews the current literature on gingipains.

INTRODUCTION

Periodontal disease is a complex process characterized by the destruction of the periodontal attachment apparatus following an inflammatory response.1 The initiation of the disease is multifactorial. The tissue destruction that occurs results from complex interactions between bacteria and the host.2 The periodontopathogen Porphyromonas gingivalis has received significant attention because it is frequently isolated from periodontal pockets and because of its ability to produce multiple proteinases, which degrade a variety of substrates.3 Gingipains are cysteine proteinases that are key virulence factors produced from P. gingivalis. There are arginine (Arg)- and lysine (Lys)-specific gingipains that are cell associated and/or released. They are referred to as Arg-gingipain (Rgp) and Lys-gingipain (Kgp).4

Enzyme purification and gene cloning studies have revealed that the 'trypsin-like' activity of P. gingivalis consists of two types of cysteine proteinases with specificity for either Arg-Xaa or Lys-Xaa peptide bonds commonly referred to as Gingipains R and K respectively.5,6

Three genes, rgpA, rgpB and kgp, encode for individual activities known as HRgpA, RgpB, and Kgp. HRgpA is a 95-kDa protein consisting of a catalytic domain in stable non-covalent association with hemagglutinin/adhesin domain or domains, while RgpB (50 kDa) has only the catalytic domain and a small C-terminal fragment of the hemagglutinin/ adhesin domain. The predominant form of Kgp in culture fluid is a 105-kDa stable noncovalent complex of a catalytic domain with hemagglutinin/adhesin domains. Taken in concern these enzymes activate or inactivate a number of human host proteins, contributing to the pathogenesis of periodontitis. A partial list includes degradation of cytokines, components of the complement system, and receptors on macrophages and T cells, thus perturbing host gingival host defenses. Mediation of the destruction of periodontal tissue is also known to occur through stimulation of gingival fibroblasts to produce matrix metalloproteinases(MMP) accompanied by direct activation of secreted but latent matrix metalloproteinases by gingipains.7,8

Gingipains degrade both collagen and fibronectin and they inhibit interactions between host cells and the extracellular matrix. Gingipains also degrade various cytokines, resulting in a disturbance of the host cytokine network. These bacterial factors contribute differentially to the progression of overall inflammatory destruction in infected periodontal tissues. Gingipains have been demonstrated to affect cytokine signaling networks and to modulate the production of proinflammatory mediators [interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor a], which may initiate tissue destruction and alveolar bone resorption.9

P.GINGIVALIS IN HUMAN PERIODONTAL DISEASe

A number of studies have revealed that these proteinases are closely associated with the periodontopathogenesis of P. gingivalis, which involves the destruction of periodontal connective tissues, disruption of host defense mechanisms, and the development and maintenance of inflammation in the periodontal pockets. Gingipains indirectly contribute to tissue damage through the activation of latent host MMPs and the inactivation of host proteinase inhibitors.9

P. g has developed strategies to ensure survival in host cells and elicit host responses that result in tissue destruction. Invaded epithelial cells are thought to provide a protective environment for the micro- organisms. The mechanism P. g uses to internalize into host cells are similar to those described for invasive enteric pathogens.10 Many recent studies have focused on P. g interactions with the invasion of epithelial cells.

Both P.g and T.denticola have several characteristics that make them prime candidates as pathogens involved in the clinical destruction of periodontal tissues:

1. They occur concomitantly with the clinical signs of periodontal destruction
2. They appear closely linked topologically in the developing biofilm
3. In vitro studies demonstrate their ability to produce a number of outer membrane-associated proteinases(eg-arginine and lysine – specific cysteine proteases, a chymotrypsin-like serine protease).11

FUNCTIONS

Certain house keeping functions of gingipains include:

- Gingipain R are considered as major processing enzyme for various cell surface protein.
- HRgpA and Kgp assist bacterial cells with an hemagglutinin/adhesion ability that is pre-requsite for colonization of host tissue.
- It has been demonstrated that gingipain R enhanced the binding of fimbriae to cultured fibroblasts or extracellular matrix proteins.

Certain pathogenic Activities of Gingipains include:

- Activation of Kallikrein/Kinin system
- Activation of blood clotting system.
- Degradation of Fibrinogen and Fibrin
- Dysregulation of complement pathway
- Desensitization of neutrophils
- Degradation of host tissues
- Dysregulation of cytokine networking system

Zhou and Windsor did12 a study and demonstrated that human gingival fibroblast cell lines from different individuals demonstrate different responses to exogenous bac-

terial stimulation in regard to their collagen-degrading ability. Treatment with P. gingivalis supernatant increased the collagen-degrading ability of several lines (aggressive phenotypes). In contrast the same treatment did not alter or decreased the collagen degrading ability of other cell types(non aggressive phenotype)

Kato T et al13 did a study and demonstrated that both the WT and KDP150 strains significantly inhibited cellular proliferation and arrested the cell cycle in the G0/G1 phase, while the expression levels of the cell-cycle regulatory molecules cyclin D and cyclin E were also decreased. In contrast, KDP136 did not show any effects. G1 arrest was also clearly induced by KDP129 and KDP133, with KDP129 being more effective. There findings suggest that P. gingivalis gingipains reduce cyclin expression and cause early G1 arrest, leading to the inhibition of cellular proliferation.

Stathopoulou et al14 did a study and concluded that P. gingivalis, through lysine gingipain, can subvert the protective host proinflammatory response by direct cytokine degradation. Changes in the crevicular cytokine profile have consequences in periodontal disease pathogenesis that should be considered in the development of diagnostic and therapeutic modalities. They also demonstrate that cytokine response differences are the result of the action of P. gingivalis proteases, with lysine gingipain being the most effective

Al Shibani et al 15 did the study on human fibroblasts. Their results showed that the collagen was totally cleaved in 12 (aggressive) of the 21 cell lines isolated from the inflamed tissues in the presence of P. gingivalis. The remaining nine cell lines (non-aggressive) cleaved only the collagen underneath the cell colonies in the presence of P. gingivalis. Of the healthy tissues, five (aggressive) of the 21 cell lines cleaved all the collagen and 16 cell lines (non-aggressive) only cleaved the collagen underneath the cell colonies in the presence of P. gingivalis. All the collagen was cleaved by an aggressive cell line and only the collagen underneath the cell colonies was cleaved by a non-aggressive cell line in the presence of Arg-gingipain. They concluded that the collagen in the wells was more readily cleaved by the inflamed than by the healthy cell lines, and the difference was statistically significant ($p = 0.0278$). Arg-gingipain gave identical results to the P. gingivalis supernatant.

Cronan CA et al 16 did a study and found that RgpB, HRgpA, and Kgp were all inhibited by chlorhexidine with Ki's in the micromolar range. For RgpB and HRgpA, the inhibitory effects of chlorhexidine were enhanced 3–30-fold by Zn(II). The chlorhexidine–Zn(II) interaction was synergistic for inhibition of HRgpA and RgpB. For Kgp, the effect of Zn(II) on chlorhexidine inhibition was antagonistic. They concluded that Chlorhexidine is an effective inhibitor of gingipains, and the inhibition of Rgingipains is enhanced by Zn(II). A mixture of chlorhexidine and Zn(II) may be useful as an adjunct in the treatment of periodontitis and in the post-treatment maintenance of periodontitis patients.

CONCLUSION

Gingipain is the main constituent of the P.gingivalis proteolytic system. Gingipains constiute group of cysteine-proteases. It is responsible for at least for 85% of the general proteolytic activity and 100% of so called trypsin like activity. Biochemically, these enzymes are members of the cysteine-proteinase family, which includes the papins, calpains, streptopains (from streptococcus), clostripain (clostridium) and various endopeptidases . gingipains are also called as Trypsin like protease Lysine specific protease of arginine specific protease,Protease I, Protease II, Gingivain, Porphypain, argingipain.

Gingipain R are the products of two related but distinct genes that have been cloned and sequenced from several strains of P.gingivalis.

To unify the nomenclature , it was suggested : rgpA and rgpB encodes Arg Gingipains whereas kgp –encodes Lys gingipain

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