VIRULENCE FACTORS OF PORPHYROMONAS GINGIVALIS

CONTENTS

INTRODUCTION

P.gingivalis is a member of the genus porphyromonas that belongs to the family Bacteriodaceae.

The family Bacteriodaceae contains many important human & animal pathogens & comprises of a large collection of genera of obligatory anaerobic, gram negative, non spore forming rods. The family was first described in 1923 with Bacteroides as the type genus. 15 genera are included under this – Bacteroides, Fuso Bacterium, heptotrichia, porphyromonas & prevotella.

Shah & Hardie (1979) and coykendall etal (1980) proposed a new species Bacteroides gingivalis, for the strains of oral origin. DNA- DNA hybridization, studies of cellular fatty acids and menaquinone compositions supported the placement of these organisms into a separate species.

The taxonomic position of P.gingivalis is firmly established within the genus porphyromonas. Recently, the definition of porphyromonas was emended to include non-pigmented & saccharolytic species. Major taxonomic revision has taken place in the genus Bacteroides over the last years. Clear genealogical differences exist between the genus porphyromonas, prevotella and Bacteroides of the family Bacteroidaceae.

Morphology of P.g:-

P.g species are nonmotile coccobacilli cells may be pleomorphie (many different shapes) ranging from medium length rods (1.5 μm) to cocci. The rods may measure 0.5-0.8 x 1.0-3.5 μm in diameter, and are non-spore forming.

Members of this species exhibit smooth raised colonies. When grown on a blood agar surface, colonies are initially white to cream colored. With time (4-8 days) these colonies darken from their edge towards the centre and a deep red to black color which correlates with the concentration of protoheme.

Structure of the cell wall:-

Cell-wall of G-ve bacteria is a complex multilayered structure so commonly referred to as cell envelope. The cell envelope consists of the inner cytoplasmic membrane, a thin peptidoglycan, attached to which is asymmetrical outer membrane. The outer membrane contains the complex lipopolysaccha ride, lipoproteins and peripheral and transport proteins. Transport proteins connect the outer membrane to the peptidoglycan and provide structural integrity to the cell envelope. Porin proteins provide a transport mechanism for the movement of selected proteins (600Da) into & out of the cell.

The surface of the outer membrane is covered by numerous thin short fimbriae. Lipopolysacharides and hemagglutinins are intimately associated with the outer membrane.

Fimbriae:-

These are thin and straight appendages associated with the cell surface & were earlier referred to as pile and shown to have importance in red blood cell agglutination. Two major classes of fimbriae have been described.

1) Those that are involved in interaction with other bacteria and mammalian cells and in adherence to soft and hard cell surfaces. These are referred to as type – specific fimbriae. (8 types identified).
2) Those that are involved in bacterial conjugation and are referred to as F- or sex-pili. These are longer and more flexible than the type specific & function in DNA transfer between the cells.

P.gingivalis produces fimbriae of between 0.5 and 1.6 μm in length and approximately 5nm in width. With one or two exceptions P.gingivalis strains contains fimbriae arranged in a peritrichous fashion over the surface of the cell. Fimbriae are composed of fimbrillin monomers which are assembled into nine units per turn of the mature hair-like tertiary structure. The native fimbriae is composed of at least 1000 protein subunits (fimbrillin subunits). Different P.gingivalis strains produce fimbriae that are

heterogeneous with respect to antigenicity. Molecular weight of the fimbrillin subunits, N-terminal sequence of the fimbrillin protein and DNA sequence. Gene encoding P.g firm A subunit has been cloned & characterized. The firm A gene is resident on the chromosome as a single copy & all P.g strains contain it.

Lipopolysaccharide:-

The outer membrane of G-ve bacteria lies external to the peptidoglyean & is attached to it by selected lipoproteins (murein lipoproteins). The outer membrane is asymmetric, the outer leaf of which contains the lipopolysaccharide. The LPS is a very large molecule (7110KDa). It is amphipathic in character. Its hydrophilic end consisting of the polysaccharide or O-specific (somatic) antigen, is exposed to the environment on the exterior surface of the outer membrane and the core region, buried within the outer leaflet which connects the O-antigen to the hydrophobic end of the molecule or lipid A. the complex LPS is embedded in the lipid portion of the outer membrane leaflet.

Hemagglutinins:-

Hemagglutination by P.gingivalis is mediated by surface-associated adhesions that have been described based on serological reactions & agglutination inhibition mediated by disbasic amino acids and sugar moieties. Intensive studies were carried out to characterize these adhesions and it was suggested that fimbriae and proteinases mediate hemagglutination. These results have been contested and both biochemical and genetic evidence have supported the view that hemagglutinins of P.gingivalis are distinct structural components. (Lepine etal 1993).

Capsule

EM examination of several P.gingivalis strains by ruthenium red staining (for the presence of acidic mucopolysaccharides) and routine lead acetate staining has revealed the presence of an electron dense layer external to the outer membrane. This electron dense layer is the polysaccharide capsule.

Chemical composition of this capsule differs between strains. Capsule of P.g 381 is shown to contain galoctose, glycose & glucosamine.

BIOCHEMICAL PROPERTIES OF P.g:-

P.g is an assachorolytic, anaerobic species & consequently colonizes sites where the oxygen tension is low but nitrogenous substrates are present in abundance.

Subgingival ecosystem provides an ideal environment for the growth of this species, as the redox potential is low here. F-nucleatum helps survival in aerobic environment. This bacterium possesses an electron transport system in which protoheme & menaquinones are the major electron carriers. It has been demonstrated that aspartate growth of P.g.

It is possible to culture P.g in the absence of exogenous menadione, hence it is assumed that P.g can biosynthesize menaquinones. Key genes involved in heme biosynthesis have been characterized, which implies that heme biosynthesis may occur.

P.g has the capacity to convert cobyric acid into a later intermediate cobinamide indicating the capacity of P.g to biosynthesize tetrapyrroles such as vit B_{12}. Studies in which cells were cultured in liquid media supplimentedwith all 20 amino acids revealed that arginine may be the primary substrate for this species. Compared to amino acids, peptides cause a marked ↑ in the growth yield of P.g. the preferential use of peptides & the enhancement of growth in the presence of proteins digested with the proteinase produced by P.g gingivain suggest a nutritional rule for this enzyme in the biology of the species.

SEROTYPES

Naito et al 1992 demonstrated 3 serotype strains of P.g (a, b & c) distinguished on the basis of autoclave extracted antigens which differentially stimulate 1L-1 secretion from macrophages. These serotype antigens are polysaccharide in composition.

Zambon et al also reported on existence of these 3 serotypes. The results from their studies demonstrated that serotype C was the most frequent serotype of P.g detected in diabetic periodontitis patients. Also significant elevations of P.g strain 381, which is representative. A serotype was demonstrated in adult & RPP patients.

Based on antigenicity of the polysaccharide K antigens, there are 6 serotype.

SEROTYPES:-

GENERIC HETEROGENEITY WITHIN P.GINGIVALIS:-

Subpopulations in p.gingivalis have been extensively studied by physiological, chemical and molecular methods.

Laliberate & mayrand (1983) demonstrated two biotypes among isolates of P.gingivalis based on the catalase test reaction & showed that human isolates were uniformly catalase- negative whereas isolates from animals were catalase positive.

Okuda etal (1988) demonstrated 5 bacteriocin types among 6 strains of P.gingivalis.

Antigenic diversity has been demonstrated amongst P.gingivalis strains.

Parent etal (1986) confirmed the catalase positive & catalase negative groups and used polyclonal antibodies against sonic extracts which resulted in recognition of different serogroups among human isolates. Group A isolates delineated from healthy patients which included the low virulence strain ATCC 33277. Group B isolates from patients with severe periodontitis which included the virulent strain w50.

Recently, the intraspecific diversity of P.g has been addressed by enzymatic and genotypic methods. Genomic restriction endonuclease analysis and ribosomal RNA gene hybridization profiles have revealed considerable diversity among strains of this species.

The most extensive study to date that have set out to investigate the heterogeneity of P.gingivalis has been undertaken by Loos etal (1993) using both multilocus enzyme electrophoresis and restriction endonuclease analysis. Allelic diversity was estimated from the electro phoretic mobilities of 16 enzymes among 92 human isolates from a wide range of geographical locations. A dendrogram of 71 electrophoretic types among these 92 isolates revealed 14 clusters at a genetic distance of about 0.28.

ADAPTATION OF P.GINGIVALIS IN RESPONSE TO ENVIRONMENTAL CHANGES:-

1) Temperature:

P.gingivalis mounts heat shock response when exposed to elevated temperature. Heat shock proteins function as molecular chaperones and are involved in protein folding and oligomerisation of structural proteins and DNA replication.

GroEL (HSP60 family) and Dnak (HSP 70 family) homolog have been described in P.gingivalis exact function & involvements of these HSP's in pathogenicity are still not known.

Amono etal (1994) have reported on the differential expression of P.gingivalis fimbrillin and superoxide dismutase in response to changes in temperature. Fimbrillin expression ↓ in response to ↑ temperature. Another study reported 11-fold ↑ in fimbrillin expression when temperature shifted from 37^0c – 34^0c. Precise biological function of this change in fimbrillin expression is not known, although it is suggested that the downregulation of fimbrillin expression may help to evade the host immune system.

Elevated superoxide dismutase activity may enable P.g to survive in environments containing high levels of superoxide produced from neutrophils.

2) P_H: stable growth of P.g is established in the range of P_H 7.5. trypsin like activity has been↑ with↑ in P_H. during initial stages of infection. P.g responds to the environmental P_H changes by producing enzymes with maximal potential for damaging host tissue.

3) Oxygen: detection of stress proteins in P.gingivalis by vayssier etal (1994) indicated that an ↑ in oxygen concentration can induce the HSP 60 like stress protein. However little is known regarding the expression of P.g genes in response to oxygen.

4) Iron & Hemin: Ability to utilize iron & hemin containing compounds for nutritional iron has been documented for P.gingivalis. Several studies have shown that P.g is capable of utilizing in vitro a broad range of hemin containing compounds such as hemoglobin, myoglobin, hemopexin, methemoglobin, oxyhemoglobin & cytochrome C. P.g has the ability to store hemin & this provides nutritional advantage for the survival of this pathogen in the iron limited environment of the healthy periodontal pocket.

Microbial assimilation of host iron can occur by several mechanisms.

→ Production of siderophores, which are low molecular weight compounds with an extremely high affinity for ferric iron.

→ Production of iron-regulated proteins, which function to acquire iron directly from the host iron-binding proteins.

P.gingivalis expresses a number of outer membrane proteins in response to iron and hemin limitation. Genco etal (1994) have established that the hemin molecule binds to the cell surface & is transported into the cell by a process that requires energy.

In addition to hemin-inducible outer membrane proteins, a number of reports have described the production of hemin-repressible proteins in P.gingivalis. These results indicate that in P.g, the expression of proteins involved in hemin transport may be positively & negatively regulated by hemin.

Chu etal (1991) demonstrated that P.g has ability to lyses red blood cells by production of hemolysin associated with the outer membrane vescicles. These vesicles function to attack and hemolyse RBC's during periods of active disease.

The storage of hemin on cell surface may be mediated by specific hemin storage proteins or may involve common hemin binding proteins. Then the iron is removed from the hemin molecule within the perioplasm. Binding of hemin may occur through both high & low affinity binding sites. Whether iron binding in P.g occurs via a single population of binding proteins or there are multiple binding sites remains to be determined.

Iron atom is essential for growth of P.g. however the specific role of iron atom in P.g growth & survival needs to be determined. Iron serves as a regulatory signal, influencing the expression of a wide variety of bacterial proteins. Hemin can influence the expression of putative P.g virulence genes. Several studies have reported that hemin limitations results in a decrease in the virulence of P.g. Growth of P.g under hemin limitation has been reported to result in reduction in the number of fimbriae on the cell surface as well as in an apparent increase in the numbers of extracellular vesicles. (mukee etal 1986). Limitation of hemin during growth of P.g ↑| trypsin like protease activity & ↓ in collagenolytic activity. Modification of the antigenicity of P.g LPS has also been reported in response

to hemin limitation (cutter etal 1996). The authors postulated that the hemin-induced LPS could serve as an outer membrane depot for hemin binding and storage during hemin-replete conditions.

Recently a putative iron transport system of P.g has been identified (Dashper etal 2004). The iron Hemin transport system (Iht) is a multicomponent inner membrane ATP binding cassette transport system associated with a Ton B linked outer membrane protein that is proposed to transport iron derived from haem. This transport system has been shown to play a role in the virulence of P.g. but there are controversial reports on this.

GENETIC SYSTEMS:-

Genetic analysis of P.g has been slow due to the lack of naturally occurring plasmids, bacteriophages & efficient genetic transformation systems. One of the first reports on gene transfer in P.g used the vector R751 to provide transfer functions & transfer of plasmid P^{VAL-1} & transposon Tn 4351 following conjugation from E coli into P.g. the high frequency of transposition together with the stability of the insertion indicated that Tn 4351 mutagenesis would be a valuable tool for examining a variety of mutations in P.g.

Mobilization of endogenous insertion sequence elements can influence the transcription & expression of gingipains. A report on a host-vector system for transformation of P.g was also described in which the plasmid vectors were first isolated from P.g. plasmid pE5-2 was employed since it could be conjugated to P.g.

Several reporter genes for transcriptional fusions have been developed & should be useful for studies of gene regulation in P.g.

In addition to reports of mobilization of endogenous insertion sequence elements there are studies which provide data supporting genomic rearrangements as a mechanism for genetic variability in P.g.

a comparison of the rgp B gene & the rgp-1 gene, revealed that there gene structures were very similar to each other. They have been generated through the duplication of an ancestor rgp gene.

In addition to gene conversion, evidence also exists for transposition as a possible mechanism for recombinational rearrangements in P.g.

VIRULENCE FACTORS

Virulence factors of P.g have been classified as

1) Factors that cause host defence evasion.
2) Factors that cause tissue destruction.

Factors that cause tissue destruction:

- Collagenase
- Fibrinolysin
- Trypsin like protease
- Gelatinase
- Amino peptidase
- Phospholipase A
- Alkaline phosphatase
- Chondroitin sulfatase
- Hyaluronidase
- Keratinase
- Heparinase
- Nuclease
- Epithelio toxin

- Fibroblast growth inhibitors
- LPS induced bone resorption
- Volatile sulphur compounds
- Indole
- Ammonia
- H_2S.

- Degradation of plasma protease inhibitors
- Degradation of iron transport proteins

2) Factors that cause host defense evasion

- Inhibition of PMN's
- Chemo taxis inhibitors
- L phagocytosis
- Lysis & intracellular killing.
- Resistance to compliment killing
- C3 & C5 degradation
- Immunoglobulin proteases
- Superoxide dismutase
- NADH oxidase
- Free radical formation

CAPSULE:-

Highly encapsulated P.g strains exhibit to ↓ autoagglutination, & more hydrophilic than less encapsulated strains. ↑ encapsulated was also correlated with ↑ resistance to phagocytosis, serum

resistance, & ↓ induction of PMN chemiluminescence. The ↓ tendency for phagocytosis is due to ↓| ability to activate the alternate complement pathway (schifferle etal 1993).

It was also demonstrated that P.g LPS alone was responsible for activating the alternative complement pathway & not the capsule. These authors hypothesized that the thick capsule functioned to physically mask the LPS, & therefore the compliment cascade could not be activated. The invading bacteria were therefore protected from opsonisation & phagocytosis.

It has been suggested that the presence of a capsule ↑ the virulence of a particular P.g strain.

The capsule was also found to interfere with the attachment of P.g to epithelial cells.

OUTER MEMBRANE PROTEINS:-

Virulent potential of several G-ve bacterial outer membrane proteins has been reported. Mihara & hold purified a 24KDa protein from outer membrane vesicles of P.g which had significant fibroblast stimulating ability. It was capable of functioning as a cell proliferation factor for a number of eukaryotic cells & of stimulating bone resorption. It was called fibroblast activating factor.

P.g also produces a 75KDa major outer membrane protein which was determined to be an immunodominant antigen of the organism. (sojar etal - 1991).

Surface proteins also help in attachment of the bacteria to hard & soft surfaces & also to each other. One of the adhesions as identified by hiratsuka etal 1992 was a 40KDa protein.

P.g produces a functional hemolysin associated with the outer membrane which causes lysis of the RBC's (chu etal 1991).

LIPOPOLYSACCHARIDE:-

Koga etal reported that LPS from P.g displayed endotoxic properties endotoxic activity is confined to lipid A, while the immunobiological activity is contained within the O-antigen.

Ogauva etal demonstrated that defined Lipid A induced 1L-1 Ra, 1L-6, 1L-8, IFN-r & Gm-CSF in monocytes. It has ability to function as chemoline inducer & antagonist. It is also a strong inducer of NK cell activity. P.g LPS was able to stimulate the production of PGE_2 from macrophages & human gingival fibroblasts. (Sismey-Durrant & Hopps-1991).

P.g LPS, especially its Lipid, is capable of stimulating the host inflammatory response indirectly via the induction of host derived cytolcine production by binding to CD14. Stimulation of macrophages with P.g LPS resulted in an 8 fold | in the secretion of Nitric oxide.

P.g LPS was not able to activate nonmyeloid cells & was significantly less effective in activating myeloid cells. The inability of P.g LPS to stimulate myeloid cells is due to its approximately 100-fold lower ability to bind LPS-binding protein. (Cunningham etal 1996).

FIMBRIAE:-

There is strong evidence that P.g fimbriae are responsible for binding the bacterium to host tissues & saliva coated hydroxyapatile. Salivary proteins statherin & the salivary praline –rich protein have been proposed as receptor proteins for fimbriae to bridge the fimbriae to tooth surface. (Amano etal 1994).

Isogai etal showed that purified monoclonal antibodies to P.g fimbriae could also inhibit the binding of the bacterium to human buccal epithelial cells in a dose dependent fashion.

In P.g fimbriae do not bind to RBC indicating that fimbriae possess host cell specificity.

Fimbriae is non-adhering strains were shorter & few in number compared to adherant strains.

Weinberg et al hypothesized that the interaction of the fimbriae with the 48KDa protein may be the first step in signaling process that helps in bacterial invasion of the cells.

The intact fimbriae, fimbrillin subunits or synthetic fimbrial peptides of P.g were also capable of eliciting several important host-associated biological responses.

All fimbrial structures are capable of stimulating production of fibroblast activating factor from HGF (Hirose etal 1990).

1L-1, neutrophil chemotactic factor, TNF from macrophages:

Fimbriae are also capable of stimulating mitogenic & polyclonal B-cell activation (ogawa etal 1991).

Fimbriae are also immunogenic eliciting both an antibody & cell-mediated response in serum & saliva.

Dickinson etal (1988) cloned and sequenced the gene encoding the fimbrillin subunit, fim A from P.gingivalis 381. The similarity of the fim A locus among the various P.g strains was examined by southern blotting & RFLP analysis. The fim A gene was found to be resident on the chromosome as a single copy gene in all P.g strains examined. The afimbriate P.g strains did not contain the fim A gene.

The fim A gene encoding fimbrillin has been classified into five variants (I to V) based on their nucleotide sequences. Majority of periodontisis patients were found to carry type II fim A organisms followed by type IV. P.g +ve healthy adults, most prevalent fim A type was type I was followed by type V. Nakagava etal 2002, cloned a new variant of fim A gene designated as type I b fim A. and this was closely associated with progression of periodontitis.

Growth of P.g at 39^0C↓ the expression of fimbrial protein & mRNA by at least 50% in comparison at 37^0C. (Xie etal).

Hemin limitation & growth in presence of 1% serum or saliva reduced transcription of the fimbrial gene by 50%.

PROTEINASES

Investigations in the laboratory have identified numerous properties of P.g that may account for its ability to cause disease & preeminent among these is the ability of P.g to elaborate a variety of proteolytic enzymes.

P.gingivalis proteases are not activated by host proteinase inhibitors. Rather evidence exists that the P.g proteases inactivate or degrade the host proteinase inhibitors.

P.g is unable to break down or degrade sugar substrates as a source of energy or to take up & use free amino acids as metabolic building blocks. Rather P.g relies on its ability to degrade proteins into short peptides that are taken in & used metabolically in the generation of energy & as source of carbon & nitrogen.

The various proteolytic enzymes include trypsin proteinases, caseinolytic proteinases & amino peptidases.

Thiol, serine & metallo proteinases:

These enzymes are either exposed at the surface of the bacterium, within the periplasmic space & in outer membrane vesicles.

TRYPSIN LIKE PROTEINASES:-

These are the most intensely studied enzymes of P.g & are reffered to as gingipains. They are classified as 1) arginine specific proteinases (Arg-gingipains) gingipain-1 (50KDa)

2) Lysine specific proteinases (Lys-gingipains) gingipain-2 (60KDa).

These are cysteine proteinases, molecular analysis revealed that Arg-gingipains are encoded by 2 genes, rgp A & rgp B, whereas the Lys-gingipain is encoded by a single gene, Kgp A.

Movement of the P.g cysteine proteinases formed in the bacterial cytoplasm to the outer membrane requires the intervention of a secretary pathway. This pathway is very similar to the IgAl secretary pathway. All these proteins are secreted via an outer membrane anchored intermediate (Zinlay 1997). The major cell associated Arginine & lysine specific proteinases of P.g have been characterized as a complex of non-covalently associated proteins designated as the Rgp A – Kg P proteinases adhesion complex formerly called prt R- prt K complex. This complex is composed of 45-

KDa Arg-specific, calcium stabilized cysteine proteinase (RgP A45), also reffered to as Arg-gingipains & 7 sequence related adhesions. (Neil m etal 2000).

Several proteases with molecular units larger than 35KDa have been reported which include calpains & clostripain. One of 75KDa molecular unit is called periodontain.

The secreted cysteine proteinase has also been shown to lyse red blood cells. This hemolytic protein was called gingivain. (shan etal 1990).

Scott etal isolated and characterized a Lys-gingivain from P.gingivalis as a thiol proteinase. Subsequently another arginine specific cysteine proteinase with molecular unit of 44KDa was isolated & given the Lame arg-gingivain is also a thiol dependent proteinase.

Effects of gingipains on host biologic systems

1) PMN phagocytosis of bacteria

Cleavage of C5 to form C5a (↑ PMN CL)

Cleavage of 1L-8 making it more active (↑ PMN CL)

Degradation of 1L-8 (↓ PMN C4)

Cleavage of PMN cell surface receptor for C5a (↓ phagocytosis)

Degradation of C3 & IgG (↓ opsonisation & phago).

2) Innate immune response

Degradation of CD14 (receptor for LPS).

3) Cytokenes: Degradation of 1L-1 β , 1L-6, 1L-8 & TNF- $\propto$.

4) Kallikrein / kinin system

Activation with release of bradykinin, a potent inducer of vascular permeability.

5) Coagulation / fibrinolysis system

Paradoxical effects involved in activation of clot formation & clot degradation.

Amino peptidases:-

Amino peptidases have been found in several members of the oral microbiota, many of which exhibit some arylominpeptidase activity. Abiko etal purified dipeptidylaminopeptidase from P.g & exposed it to type I collagen cleaving a glycylproply dipeptide from the collagen protein.

Grenier & mc bride have purified a gry-pro amino peptidase from P.g outer membrane. They were successful in localizing their amino peptidase activity to the surface of P.g.

Caseinases:- Caseinases hydrolyze the protein casein & exist in P.g as at least 3 isoenzymes. They are active against salivary lysozyme & insuten chain-B. these three caseinolytic proteinases are called pase- A, -B, & -C.

Collagenase:- P.g produces collagenases (metalloproteases) which might contribute to the destruction of the supporting tissues of the teeth, however several studies have demonstrated that the collagenolytic activity involved in periodontal tissue destruction predominantly involves the host derived enzyme. Several investigators have postulated that the P.g collagenase may participate with host derived collagenase in destruction of gingival collagen.

Birkedal- Hansen etal & Vilto etal portulated that a proteinase from P.g may directly induce production of collagenase from human gingival fibroblasts.

Serine proteases

Two serine proteases have been isolated from P.g: prolyl tripeptidase (ptpA) & prolyl dipeptidyl peptidase IV.

Functions of P.g Proteases:-

1) Adherence to host cell tissues.
2) Support growth of .g

3) Inhibiting selected host defence mechanisms
4) Involved in direct tissue destruction
5) Upregulation of vascular permeability (hence | GCF flow)
6) Degrading 3 & interfering with phagocytic events.
7) Degradation of host cell membranes to uncover hidden binding sites or cryptilopes
8) Capable of depolymerizing human plasma fibronectin.
9) Rapid degradation of intracellular fibrinogen matrix.
10) Degradation of collagen, casein, gelatin, fibrin, immunoglobulins, compliment factors, proteinase inhibitors, iron transport proteins.
11) Degrading type I & IV collagen, IgG, fibronectin.
12) Cause platelet activation.

INVASION:-

Clinical studies have clearly demonstrated the ability of P.g to infiltrate oral epithelium (Saglie etal 1986). In vitro studies have confirmed bacterial adhesion to & invasion of gingival epithelial cells for P.g. Internalization of P.g was preceded by formation of micovilli & coated pits on the epithelial cell surfaces.

P.g was capable of replicating with in the epithelial cells.

Invasion is an active process requiring both bacterial & epithelial cell energy production & was sensitive to inhibitors of microficaments & microtubules, demonstrating that epithelial cell cytoskeletal rearrangements are involved in bacterial entry.

Intracellular counts & during the first 24 hours & showed a 3 fold increase during the second day, indicating intracellular multiplication. The ability of P.g to persist & multiply within epithelial cells may be an important pathogenic mechanism in periodontal disease. (madianos etal 1996).

It has been shown that the release of calcium from an intracellular store with the subsequent | in cytosolic Ca^{2t} could be involved in the invasion process. Thus the invasion may be correlated with the activation of Ca^{2t} dependent host cell signaling systems (Izutsu etal 1996).

Sandros et al demonstrated that P.g internalization involved a receptor – mediated endocytosis pathway with activation of tyrosine phosphorylation in signal transduction during invasion.

Thus, the bacterial – receptor interaction & the phosphotyrosine- dependent intracellular signaling trigger an internalization process involving re-arrangements of cytoskeletal microtubules.

PMN functions:-

P.gingivalis affects PMN functions (scrags etal 1995)

→ It affects cell surface receptor expression.

→ ↓ chemotaxis & phagocytosis

→ ↓ Super oxide production and bacterioidal activity

→ there is loss of F-actin.

→ ↓ Polarization of cells

A recent study showed that LPS from P.g can ↑ mRNA expression of supervilli in PMN's. It is an F-actin binding protein associated with actin filaments and plasma membrane controlling cytoskeletal actin filaments. The ↑ could cause morphologicalchanges in cytoskeleton leading to altered neutrophil function. Random migration & chemotaxis

Effects on Fibroblasts:-

Vesicles of P.g were shown to be cytotoxic to fibroblasts (Gvigand etal 1995). There was 4 in percentage of cells in S phase, hence ↓ in proliferation. Fibroblasts remained blocked in the G0-G1 quiestant phase.

Effects on Junctional Epithelium:-

Papapanou etal 1994 demonstrated that P.g is able to invade consecutive epithelial cell layers and multiply intracellularly 8 hours were enough for P.g to advance beneath the superficial layers. Whereas an epithelial cell takes 5-7 days to reach superficial strata from basal layer during keratinisation. Hence P.g is capable of counteracting the defensive role of epithelial desquamation.

IMMUNE RESPONSE

Cell mediated immune response

1) PMN responses:-

Some severe forms of periodontitis may be correlated with intrinsic or extrinsic PMN defects, it may be proposed that certain PMN-mediated defensive mechanisms are of paramount importance in regulating the crevicular levels of P.g.

In GJP, the predominant isolation of P.g from subgingival plaque has been associated with PMN alterations.

P.g enzymes have been shown to degrade TNF- $\propto$, one factor which helps in priming of PMN's (Calkeins etal 1998).

In vitro incubation of P.g with epithelium impedes the transepithelial migration of PMN's in response to N-fmlp & 1L-8.

P.g expressing copious extracellular polysaccharide was found to be more resistant to phagocytosis & intracellular killing than the one with less polysaccharide. (Genco etal 1995).

Opsonization of virulent strains of P.g requires both antibody & complement, whereas nonvirulent strains can be opsonised without the antibody (Anderson etal 1995). LPS from P.g is capable of priming PMN's and depends on the growth of P.g in the presence of hemin (Champagne etal 1996). Gingipain K & R have shown to activate oxidized C5 & lead to the generation of C5-derived factors that can stimulate PMN polarization, chemotaxis & secretion.

2) monocyte/ macrophage responses:-

Fujiwara etal showed that LPS from P.g enhanced 1L-1 production by macrophages.

Lipid A of P.g is reported to have similar potency of LPS in stimulating 1L-1Ra, 1L-6, 1L-8, Gm-csf & IFN- $\propto$ secretion from human peripheral blood monocytes. (Ogawa etal 1996).

However LPS/ Lipid A of P.g demonstrate lesser activity compared to that from other G-ve enterobacteria. The unique fatty acid composition & the lack of a PO_4 group in the core structure of P.g LPS may contribute to the lower activity. Ogawa & Hamada reported that fimbrail protein from P.g stimulates monocyte chemotaxis. Katawa etal reported that fimbriae stimulate bone resorption through monocyte cytokine release.

3) Lymphocytic responses:-

A purified 75-KDa protein of P.g has been shown to exhibit potent polyclonal activation of B cells. (watanabe etal 1996).

Gemmell etal have studied the effect of P.g on the T-cell receptor repertoire & have found that there were no differences in the mean percentage of CD4 or CD8 cells expressing T-cell receptor $\propto \beta$ regions after stimulation with P.g outer membrane antigens. There was however a trend towards a | in % of positive CD4 & CD8 T cells after culture with the antigen.

P.g stimulated mononuclear cells produce significantly lower levels of 1L-2, therefore the THL response was proposed to be down regulated in response to P.g.

4) Role of Apoptosis: - cell proliferation P.g LPS has been reported to induce apoptosis before a specific immune response (Isogai etal 1996). Apoptosis was associated with the expression of IgA on B cells and of CD5 on T cells. TNF- $\propto$ may be a mediator of apoptosis. It was suggested that P.g can potentially induce apoptosis to eliminate the early non specifically activated lymphocytes.

Harris etal 2002 suggested that apoptosis of T cells induced by P.g was associated with an ↑ in histone H_4 acetylation.

HUMORAL IMMUNE RESPONSES

There are number of important concepts that have driven the scope of research in antibody studies of P.gingivalis

1) Based upon the humoral response, P.g is probably a causative agent in periodontal disease.
2) This humoral response is probably protective.
3) Diseased & healthy individuals can be distinguished in terms of their antibody response to this organism.
4) Differences in the antibody response characteristics are noted in different periodontal disease classifications.

Various investigations have identified elevated systemic antibody responses to P.g in periodontal disease (Ebersole 1990) while IgG is a distinctive immune response to P.g elevated levels of both Igm & IgA isotypes have also been shown.

Elevated antibody responses in periodontitis patients have also been abserved to a 75KDa protein that is a surface antigen, appears to be associated with the fimbriae , a heamaglutinin & a hemagglutinating adhesion antigen.

Increased leads of antibodies to HSPs & LPS of P.g have been demonstrated | AB levels to trypsin-like protein produced by P.g (Ismaiel etal 1988).

Ig Subclasses:

Early studies of IgG subclass antibody in periodontitis noted significant elevations in IgG2, IgG1 & IgG4 to P.g in G-EOP & adult periodontitis patients.

Farida etal have also demonstrated IgG1, IgG2 & IgG4 antibody levels to P.g to be elevated in severe periodontitis group.

They found the subclass order in both patients & controls to be IgG2> IgG3> IgG1> IgG4.

The relative predominance of IgG2, a subclass that lacks strong complement fixation & opsonic properties & the low avidity of patient anti- P.gingivalis antibodies suggested that the humoral response to infection may be ineffective in clearing this organism.

Ogawa etal 1990 have shown IgG2 as the principal subclass response to LPS from P.g & IgG3 was the predominant response to fimbriae from the microorganism.the subclass responses to RgpA- KgP (proteinase – adhesion complex) revealed that subclass distribution was IgG4> IgG2 >IgG3 = IgG1 (Neil.m etal 2000).

ROLE IN PERIODONTAL DISEASE

Occrance & prevalence:-

Most authors agree that periodontally healthy children & actolescents horbor few or no P.g in the subgingival microbiota (Kononen etal 1992, Ashimo to etal 1996). However based on DNA probe analysis Bimstein etal described subgingival P.g in many healthy children.

P.g has been described in 37-63% of localized juvenile periodontitis patients (Lope3 - etal). However the organism is rarely found at the debut of the disease & tends to comprise only a small part of the microbiota in early stages.

Adults having a healthy & minimally diseased periodontium reveal subgingival P.g in less than 10% of sites (Dimurro etal) on the other hand 40-100% of adult periodontitis may yield the organism (papapanou etal).

P.g comprises a higher proportion of the subgingival microbiota in deep than shallow packets. (Ali etal 1996, kamma etal 1995).

Ethnic distribution:-

Umeda etal found P.g to be more prevalent in the subgingival microbiota of hisponics (oddo ratio 6.1), African, Americans (OR – 2.7) & Asian – Americans (OR – 5.4) than in that of caucascans.

Ellwood etal showed that subgingival P.g to occur 3-4 times more frequently in indo-pakistani children than in children of caucasion.

Effect of periodontal treatment on subgingival P.g:-

SRP may cause temporary 4 in levels of subgingival P.g but is not capable of eradicating the organism from subgingival sites. (Flemming etal 1998).

Location of P.g in inaccessible areas, such as furcations or base of pockets is probably responsible for the failure of mechanical therapy to remove the organism.

Surgery aimed at eliminating periodontal pockets provide predictable suppression of P.g. (Rosenber etal 1993 & Monbelli etal 1995).systemic antibiotic therapy in combination with nonsurgical therapy may not ensure subgingival eradication of P.g. Neither is topical antimicrobial therapy very useful in eliminating P.g from deep periodontal pockets.

The most effective control of periodontal P.g seems to be achieved by employing a combination of surgery, systemic antibiotic therapy and good oral hygiene.

P.g was related to implant failure in a number of studies (Backer etal 1990, mombelli etal 1987).

Regeneration of periodontal connective attachment is contingent upon the absence of P.g. P.g is a major detriment to periodontal regeneration.

Zarkesh etal (1999) recovered P.g from barrier membrane treated site that lost clinical attachment & from a barrier membrane treated site that showed no attachment gain. They also described a method of coating membranes with Td. Compared with non-coated, the coated membranes permitted less P.g colonization & more clinical attachment gain.

Giano etal – 1996 examined colonization of 6 different bioresorbable and non-resorbable membranes for periodontal regeneration by P.g.

Biofix → After 48 hrs bacteria were seen on inner surface, mainly adherent to fibrils & in interstitial spaces.

Goretex → After 48 hrs large. Numbers of cells were detected on both fibrils of external & internal sides.

Guidor → very few cells attached to both external & internal surface.

Paroguide → Strongly adhered to external surface.

Resolut → Microcolonies were present on rough areas of fibrils.

Vicryl → Widespread all over external & internal surface. P.g has strong affinity for polygloctin 910 matrix.

It has been shown that P.g reduces mitogenic & chemotactic responses of human PDL cells to PDLF (matsuda - 1996) and so also the effect of EMD (Inaba etal - 2004).

Transmission:-

Von troil Linden etal (1995) demonstrated transmission of P.g among spacises in a case – control study. 10 cases & 10 control groups. P.g was found in 7 spouses of diseased probands, but only in 2

spouses of the healthy ones. Vander velden etal 1993 examined the effect of sibling relationship & showed that P.g aggregated within the families.

P.g in non-oral infections:-

P.g has only occasionally been recovered from non-oral infections. A few reports have implicated it in non-oral diseases.

Pancholi etal isolated P.g from 2 patients with chronic suppurative otititis media, from 2 pts c lung abscesses & from a Pt c gas gangrene. Matto etal recovered P.g from intra-abdominal infections. It has been occasionally been involved in chronic sinusitis, paranasal mucopyets, peritonsillar abscesses, pleuro-pulmonary infections, tonsillitis & thoracic empyema.

VACCINE DEVELOPMENT

Vaccines for periodontitis may be expected to be administered to large numbers of people & as periodontal disease is not seen as life threatening; even a low level of vaccine toxicity may be considered unacceptable.

However recent reports demonstrating a link between CVS & other systemic diseases & periodontal disease suggests that the latter may contribute in some way to systemic sequelae of morbidity & mortality.

Strategically to limit the transmission or dissemination of periodontopathic bacteria it would appear advantageous for an effective vaccine to induce immunity at 3 levels.

1) Local mucosal secretory Ig A
2) Local draining lymphnodes
3) Circulating specific T & B cell responses.

The primary infection & pathogenic mechanisms in mucosal infectious processes include activities such as microbial attachment, colonization, tissue & cell invasion & localized toxin release. Thus the principal efforts of vaccination against these types of pathogens should be to elicit localized immune responses, including immune memory that can prevent those vivulent determinants.

For the development of a vaccine, the target bacteria & antigens need to be identified. The more complex an ecosystem, the more likely that niches will overlap, so that lose of one species is compensated by others. Main concern is the species "Kingpin", the loss of which results in the collapse of entire ecosystem. However kingpins are often difficult to identify. It has been emphasized that some prime moving event that causes the loss of a plaque kingpin. But it is not clear what the prime mover is & is there a kingpin microorganism in dental plaque.

The idea of a vaccine to control oral bacterial infections was developed in the early part of this century. Autogenous vaccines, pure cultures & mixed stock vaccines were all employed at various times. Various strategies have been tried for periodontitis as well.

1) Attanuated & inactivated bacterial vaccines:-

Studies have documented production of serum antibody, which correlated with immune protection from the virulence properties of P.g & A.a [Evan etal 1992 & Taubman 1983]. Additional studies have demonstrated that active immunization of mise or rate has ability to alter disease manifestations of periodontitis.

Wikesjo etal showed that subgingival irrigation with H_2O_2 can suppress A.a & the effect can last for 6 months.

2) Surgical Treatment:-

Treatment of mod-sev periodontitis with open flap debridement showed decline of A.a +ve sites from 53.3% to 13.3% (Danser etal).

3) Adjunctive Antibiotics:-

Flemming etal treated periodontitis either with SRP alone or SRP, metro+Amox and supragingival irrigation with chx. A.a +ve sites declines from 100% to 92% in case of SRP & from 100% to 50% in case of combined therapy.

Subgingival prevalence:-

Most studies on the subgingival presence of A.a have examined only one or a few sites in the dentition & therefore may have underestimated the periodontal occurance of the organism. As shown by chistersson etal (1992) the detection of periodontal A.a may require the sampling of a minimum of 25 random sites.

CONCLUSION

This commensal, opportunistic pathogen has evolved with numerous mechanisms and strategies to effectively compete in the complex environment of the host subgingival pocket. These biomolecules / structures enable adherence to host tissues and multiple bacteria that establish subgingival biofilms. Additionally, the asaccharolytic metabolism has evolved to compete effectively for nutrition and micronutrients in the changing host environment, as well as providing metabolic end-products to help sustain the complex biofilm organization. Finally, P. gingivalis demonstrates multiple components, particularly its cysteine proteases (i.e. gingipains) that alter host cell and tissue functions, and decrease, or even, negate host protective responses.