# KEY PATHOGENS OF PERIODONTAL DISEASE

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# INTRODUCTION

# ORAL CAVITY AND MICROBES

- The human fetus inside the uterus is sterile , but after passing through the birth canal, fetus acquires vaginal and faecal microorganism.
- Within hours after birth, the sterile oral cavity becomes colonized by low numbers of mainly facultative and aerobic bacteria.
- 2nd day-anaerobic bacteria develops.

# ORAL CAVITY AND MICROBES

- 6 basic ecosystems / niches :
  1. Intraoral supragingival hard surfaces (teeth, prosthesis , restorations )
  2. Periodontal pocket , peri implant pocket
  3. Buccal epithelium , palatal epithelium, floor of the mouth
  4. Dorsum of the tongue
  5. Tonsils
  6. The Saliva

# PERIODONTAL PATHOGENS

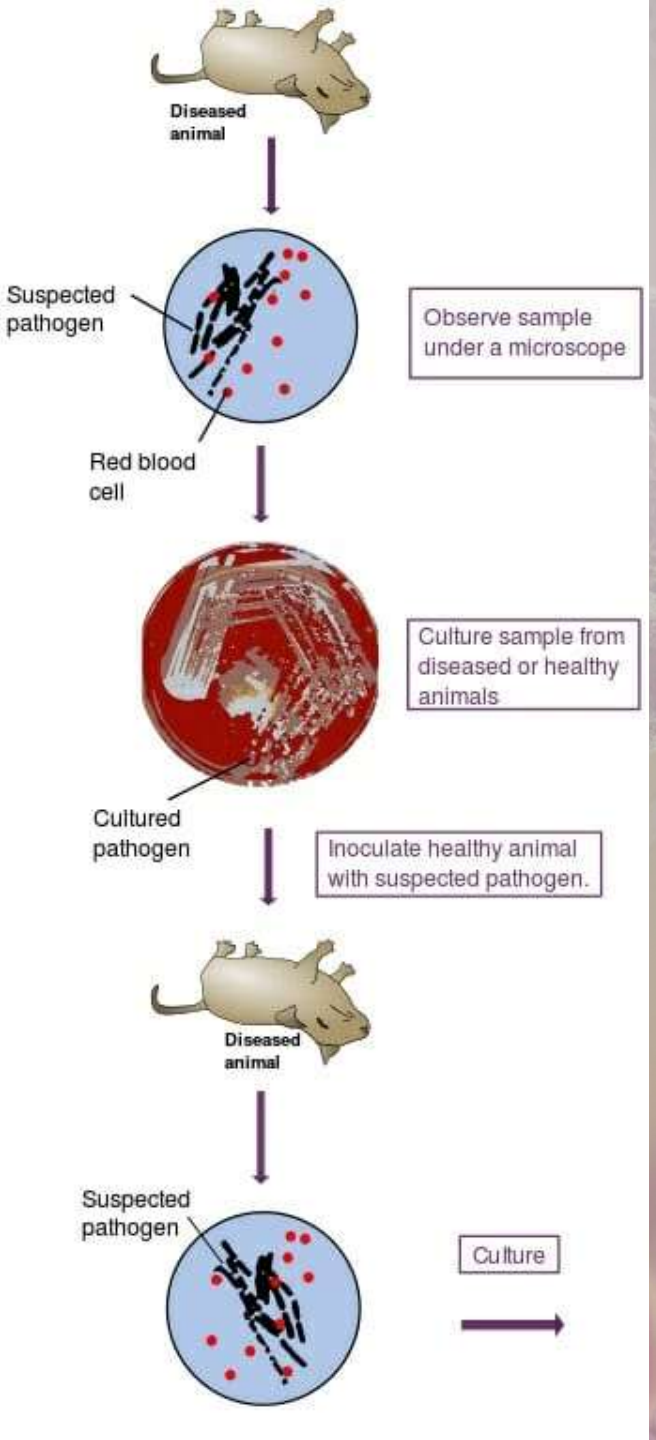
# Koch's Postulates:

① The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

② The microorganism must be isolated from a diseased organism and grown in pure culture.

③ The cultured microorganism should cause disease when introduced into a healthy organism.

④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.



# KOCH'S POSTULATES

- Applicability of koch postulate in periodontal disease
- In periodontitis 3 main problem existed

  1.Inability to culture all the organism

  2.Difficulties in defining and culturing sites of active disease

  3.Lack of animal model system

# SOCRANSKY'S CRITERIA

- Sigmund Socransky, a researcher at Forsyth dental center, Boston, proposed criteria by which periopathogens can be identified.

➢Must be associated with the disease, evident by organism must be found in relatively high numbers in proximity to the periodontal lesion

➢Must be eliminated or decreased in sites that demonstrate clinical resolution of the disease with treatment.

# SOCRANSKY'S CRITERIA

- Must demonstrate a host response, in the form of an alteration in the cellular or humoral immune response.
- Must be capable of causing disease in experimental animal models.
- Must demonstrate virulence factors responsible for enabling the microorganism to cause destruction of periodontal tissues.

# IN HEALTH AND DISEASE

# BACTERIA IN ORAL HEALTH

- **<u>FACULTATIVE GRAM-POSITIVE BACTERIA SUCH AS :</u>**
  - ✓STREPTOCOCCUS SPECIES
    - S.Sanguis (PRODUCES H2O2 – LETHAL TO A.A)
    - S. Mitis
  - ✓ACTINOMYCES SPECIES
    - A.Naeslundii
    - A. Viscosus

# BACTERIA IN ORAL HEALTH

- **GRAM NEGATIVE SPECIES :**
  - Prevotella intermedia
  - Fusobacterium nucleatum
  - Capnocytophaga
  - Neisseria
  - Veilonella species (increased in inactive sites , prevents colonization and proliferation of pathogenic organisms )
  - Few spirochetes and motile rods

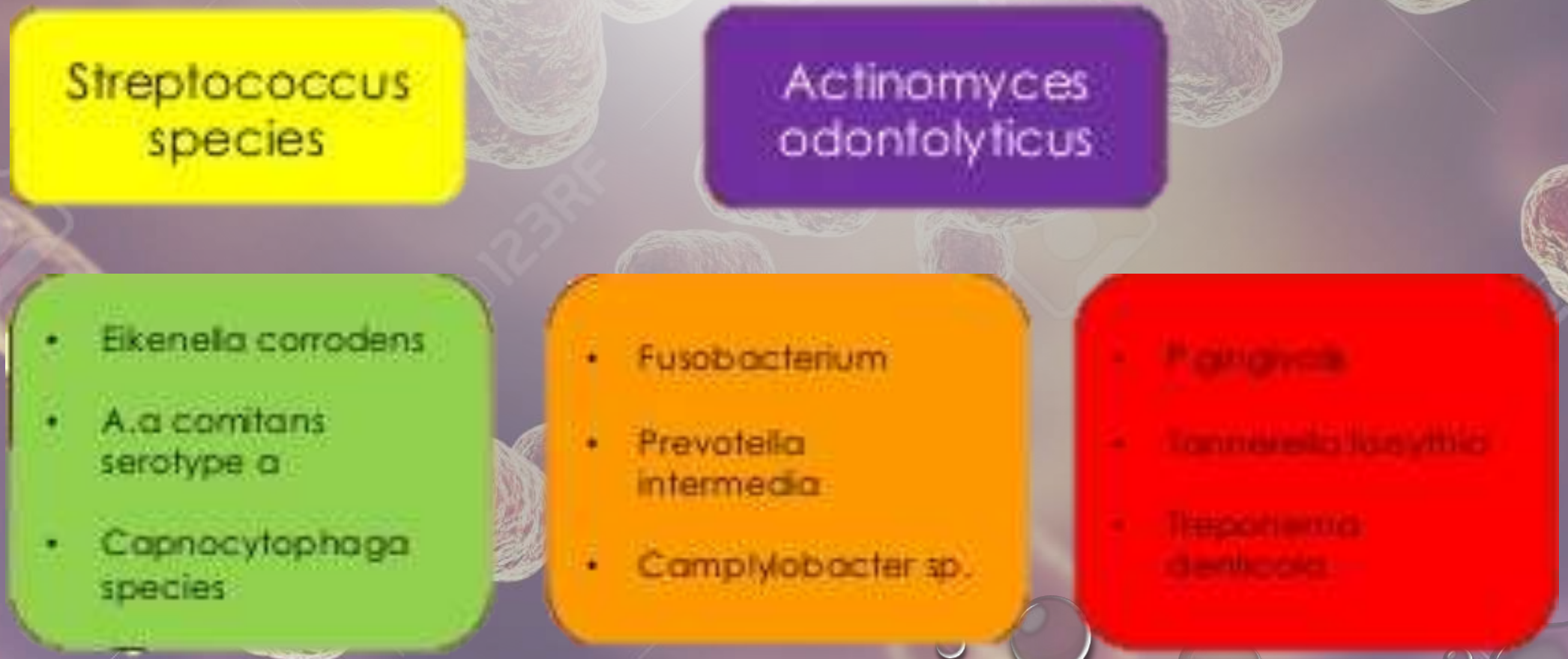
# MICROBIAL SHIFT

# SHIFT FROM HEALTH TO PERIODONTAL DISEASE

- In the process…..
  - ✓Gram positive → Gram negative
  - ✓Aerobic → Anaerobic
  - ✓Facultative → Obligatory
  - ✓Fermentive → Proteolytic
  - ✓Non-motile → Motile

# BACTERIAL COMPLEXES

- As described by Socransky et al 1998:
- 5 complexes:



# BACTERIAL COMPLEXES

## PRIMARY COLONIZERS:

- ✓ Streptococcus gordonii
- ✓ Streptococcus intermedius
- ✓ Streptococcus mitis
- ✓ Streptococcus oralis
- ✓ Streptococcus sanguinis
- ✓ Actinomyces gerencseriae
- ✓ Actinomyces israelii
- ✓ Actinomyces naeslundii
- ✓ Actinomyces oris
- ✓ Aggregatibacter actinomycetemcomitans serotype A
- ✓ Capnocytophaga gingivalis
- ✓ Capnocytophaga ochracea
- ✓ Capnocytophaga sputigena
- ✓ Eikenella corrodens
- ✓ Actinomyces odontolyticus
- ✓ Veillonella parvula

# BACTERIAL COMPLEXES

## SECONDARY COMPLEX:

- ✓ Campylobacter gracilis
- ✓Campylobacter rectus
- ✓Campylobacter showae
- ✓Eubacterium nodatum
- ✓Aggregatibacter actinomycetemcomitans serotype b
- ✓Fusobacterium nucleatum spp.
- ✓Parvimonas micra
- ✓Prevotella intermedia
- ✓Prevotella loescheii
- ✓Prevotella nigrescens
- ✓Streptococcus constellatus
- ✓Tannerella forsythia
- ✓Porphyromonas gingivalis
- ✓Treponema denticola


A naeslundii 2
(A viscosus)
V parvula
A odontolyticus
S mitis
S oralis
S sanguis
Streptococcus sp.
S gordonii
S intermedius
C gracilis
C rectus
P intermedia
P nigrescens
P micros
F nuc vincentii
F nuc nucleatum
F nuc polymorphum
F periodonticum
S constellatus
E nodatum
C showae
P gingivalis
T forsythensis
T denticola
E corrodens
C gingivalis
C sputigena
C ochracea
C concisus
A actino. a

# VIRULENCE FACTORS OF PATHOGENS

- Virulence is defined as the ***relative capacity of a microbe to cause disease*** **(Slots, 1999)**
- **Poulin and Combes (1999)** defined the concept of virulence in terms of the “virulence factors”, which are ***molecules or components from a microbe that harm the host.***

# VIRULENCE FACTORS OF PATHOGENS

1. Factors that promote colonization (adhesins)
2. Toxins and enzymes that degrade host tissues
3. Mechanisms that protect pathogenic bacteria from the host.

# AGGREGATIBACTER ACTINOMYCETEMCOMITANS

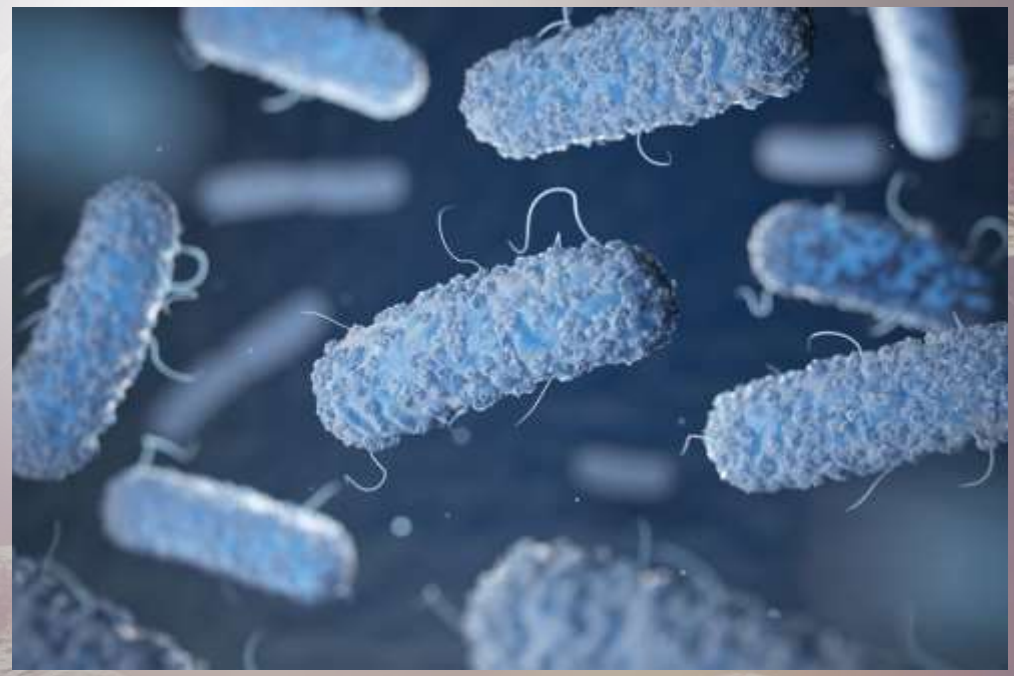
# INTRODUCTION

- Aa member Actinobacillus
- Actes- ray
- Mycetes- fungus
- Comitans- common with
- Klinger et al in 1912 – cervicofacial actinomycotic lesion
- Initially designated , Bacterium actinomycetemcomitans.
- Lieske in 1921 – Bacterium comitans
- Topley et al in 1929 – finally designated as AA

# HISTORY

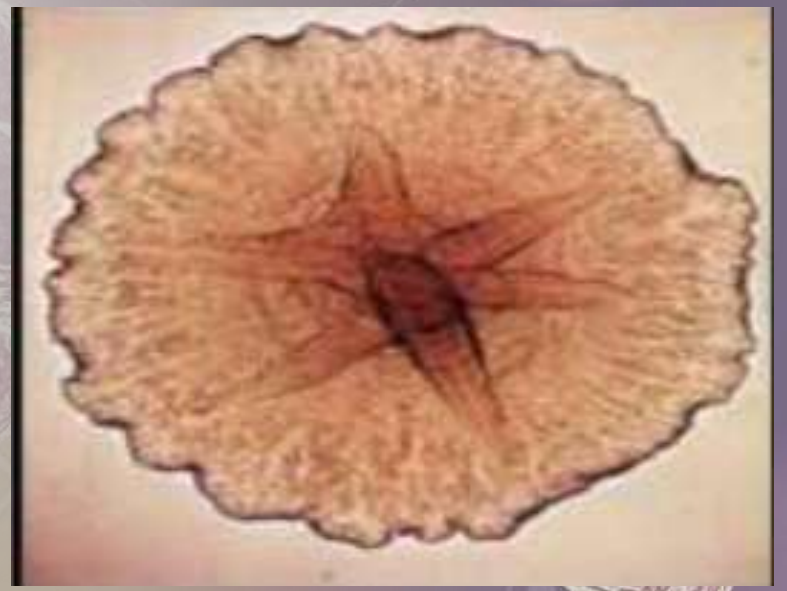
- Home- 1951- AA cause disease in humans.
- 1976- Newman, Socranksy- AA to juvenile periodontitis.
- 1979- Tsai AA leuckotoxin
- Page et al 1991 Perry et al 1996, Kaplan et al 2001- serotype A-F
- In 2004 , Roe et al – identified the complete A.A genome
- In 2006- studies have shown a phylogenetic similarity of A. Actinomycetemcomitans and haemophilus aphrophilus, H. Paraphrophilus, and H. Segnis, suggesting the new genus ***aggregatibacter.***

# GENERAL CHARACTERISTICS



- Fastidious
- Facultative anaerobe
- Nonmotile
- Non sporing
- Non hemolytic
- Small gram-negative rod, 0.4–0.5 lm x 1.0–1.5 lm in size
- Oxidative positive and catalse positive.
- Capnophillic
- The detection frequency of AA in indian population – 61%

# COLONY CHARACTERS:



- Culture- blood or chocolate agar
  i. Irregular edged, domed, central wrinkling,
  ii. Star like
  iii. Reduce nitrites to nitrates
  iv. Selective medium- Tryptic Soy–Serum–Bacitracin–Vancomycin Agar (contains 10% horse serum, 75 mg/l bacitracin and 5 mg/l vancomycin)
  v. The presence of these antibiotics suppresses the growth of gram-positive bacteria

# SEROTYPES

- Three major phylogenetic lineages comprise (antigenic composition of A.A )
  i. serotype b strains
  ii. serotype c strains
  iii. serotype a, d, e and f strains
- Actinomycetemcomitans serotypes appears to vary according to the persons geographical location and their ethnicity.
- Serotype A → stable in the host over time

# VIRULENCE FACTORS

- Adhesion to cells
- Invasion of cells
- Ability to evade host defence mechanisms, including the innate and acquired immune systems.
- Enzymes such as proteases, which can directly damage host tissues.

- HOLT AND EBERSOLE IN 2005

# ADHESION

- Long distance adhesive systems of fimbriae and flagella to fibrils
- Short-range adhesive proteins attached to the bacterial cell wall
  1. Tight adhesion A-G operon
  2. Fimbriae associated protein
  3. Colony proteins
  4. Adhesins
  5. Omp-100
  6. Collagen-binding proteins

# A] TIGHT ADHESION A-G OPERON

- TAD A-G
- Responsible for tight adhesion
- Comprises seven adjacent genes A – G
- Mutations – low levels of fimbrie expression and influence release of leukotoxin

# B] FIMBRIAE ASSOCIATED PROTEIN

- Gene inactivation → failure to produce fibrils and a loss of adherence.
- Mutations → losses its ability to form micro- colonies
- Mutants of FLP-2 → lower levels of fimbriae expression.

# C] COLONY PROTEINS

- Rough Colony Proteins:
- The RCPA, B AND C PROTEINS → OUTER MEMBRANE
- RCPA forms a multimeric outer-membrane secretion channel (a so-called secretin) for generation of the fimbriae

# D] ADHESINS

- Most prevalent bacterial adhesins is the receptor for the host glycoprotein fibronectin → COME1 gene
- Binds to a unique site in fibronectin – the FNIII9-10 domain
- Binds to the cell-surface *integrin α5β1* on the cell being invaded, with the bacterium entering by ***receptor mediated endocytosis***

# E] OMP100

- OMP100 Is homologous to the family of adhesins
- This protein is randomly localized on the bacterial outer surface
- An antibody to the protein was able to inhibit binding (and invasion) of A. A to human gingival keratinocytes.
- Inactivation of the gene decreased adhesion and invasion by 60%.

# F] COLLAGEN-BINDING PROTEINS

- MINTZ identified a gene encoding the protein EMAA (EXTRACELLULAR MATRIX PROTEIN ADHESIN A), A COLLAGEN-BINDING ADHESIN.
- EMAA Is the largest oligomeric coiled-coil adhesin protein (202 kda) electron microscopic examination of A.A identified antenna-like protrusions on the bacterial surface.
- Such structures are absent in EMAA mutants and collagen binding is decreased

# BIOFILM FORMATION BY A.A

- POLY-N-ACETYL- GLUCOSAMINE
- Mediates intercellular adhesion and attachment of cells to abiotic surfaces.
- It offers a high degree of protection of A. Actinomycetemcomitans and other bacterial biofilms against detergents like sodium dodecyl sulfate and macrophage killing.

# INVASION

- A.A was the first invasive periodontopathogen to be reported.
- Invasion process is a rapid mechanism involving the formation of cell- surface craters or apertures, with bacteria appearing in the host cell cytoplasm within 30 min.
- Invasion was associated with protrusions from the host cells that formed connections between cells and harboured a. A and entry of A.A is rapidly followed by cell division.
- Bacteria interact with the intracellular actin cytoskeleton to invade and move through cells ´

# TOXINS

(i) RTX (REPEAT IN TOXIN) LEUKOTOXIN

(ii)CYTOLETHAL DISTENDING TOXIN (CDT)

(iii)TOXIN ENCODED BY CYTOTOXIN-ASSOCIATED GENE E (CAGE)

# LEUKOTOXIN

- Characteristic calcium-binding motif that is repeated in the carboxy terminal of such proteins
- Gene PTSH is required for LTXA secretion.
- Strains such as JP2 produce very large amounts of toxin.
- Associated with severe forms of periodontitis
- Positive association between the presence of this clone and the occurrence of early-onset periodontitis (EOP) (Haubek et al).
- advanced stages of the disease than patients without the clone - Haubek et al

# LEUKOTOXIN

- SECRETION OF LTX:
- Microvesicles in outer membrane
- Morphogenesis protein C (MORC).
- Inactivation of MORC results in the changing from an irregular to a flat profile of outer membrane of A.A and failure to secrete LTXA.
- Studies have suggested that human serum can cause the release of LTXA from A.A and that secretion is blocked by the presence of free iron

# LEUKOTOXIN

- ROLE OF LTX:

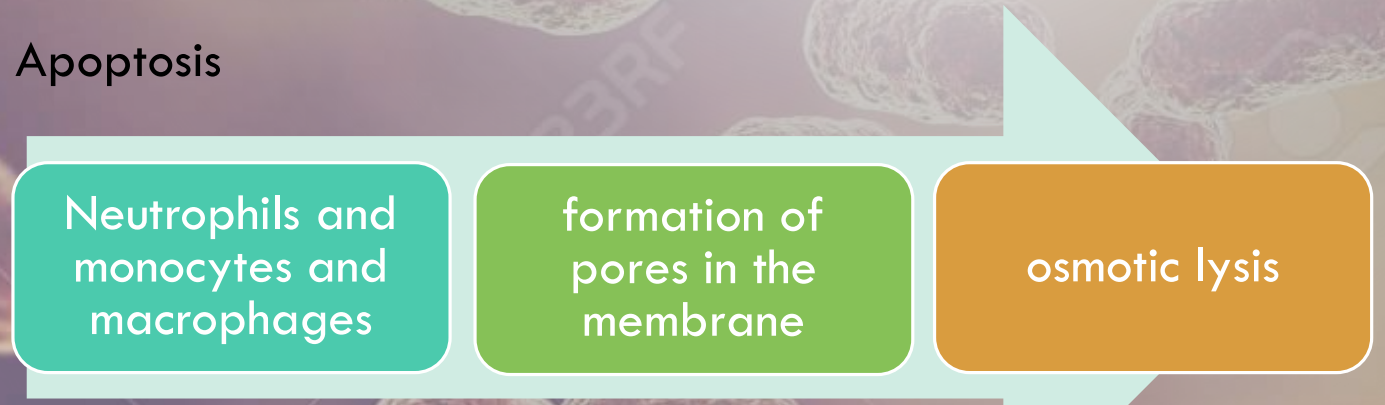
i. The bacterium inducing pro-inflammatory factors and tissue-damaging agents

ii. Inhibition of the killing actions of the key anti-bacterial components of immunity (phagocytes)

iii. Protection of the bacteria from immune-mediated killing

# LEUKOTOXIN

- The mechanism of leukotoxin action :
  - Necrosis
  - Apoptosis




- Prolonged exposure of lymphocytes and nk cells to ltxa results in apoptosis. ´ Lower concentrations lead to apoptosis while higher concentrations lead to necrosis

# CYTOLETHAL DISTENDING TOXIN

- Cell cycle blocking bacterial toxin
- Block cell division in G2
- Toxin enter cell to nucleus to exerts its effect
- Bind to cholesterol within cell membrane removal of this lipid result in loss of activity of toxin.
- Aa block T cell proliferation with CDTB along with CDTA and C

# CYTOTOXIN-ASSOCIATED GENE E (CAGE)

- Identified by TENG & HU
- The protein was shown to induce changes in cells similar to those reported for the H. Pylori protein
- Associated cytotoxin associated genes A and E (CAG A/E)
- Cause cellular alterations such as increased cell proliferation, motility, apoptosis and morphological changes

# CELLULAR MECHANISMS RESPONSIBLE FOR BONE DESTRUCTION

- The various components are :
- Lipopolysaccharide -chaperonin 60 (hsp60)
- Capsular-like polysaccharide
- Cell-wall components of A. Actinomycetemcomitans has osteolytic signals.
- These have included lipopolysaccharide and cell-surface capsular-like polysaccharide.

- Inhibit osteoblast proliferation
- Synthetic activity
- Activation of bone resorption
- The induction of osteoclast proliferation and activation

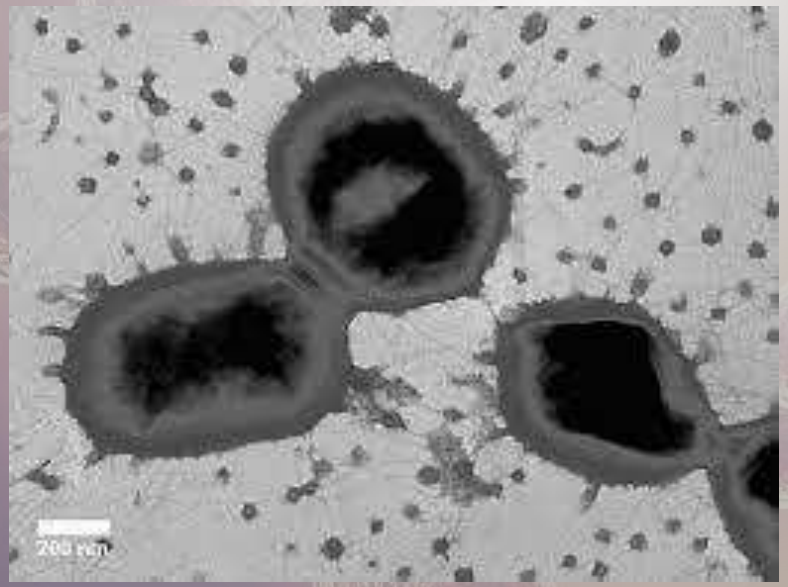
# PORPHYROMONAS GINGIVALIS

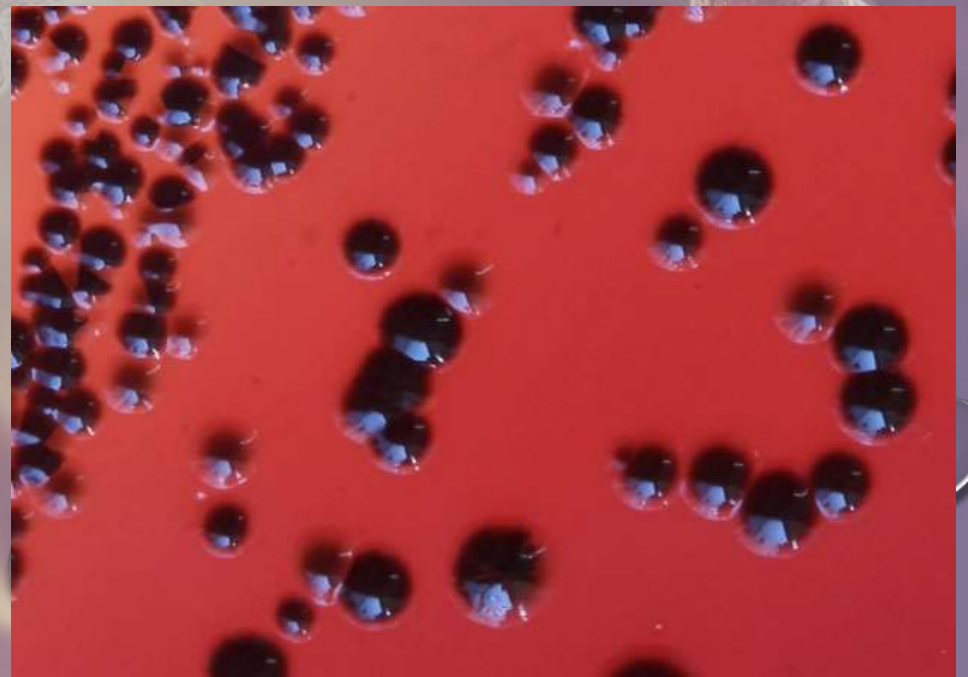
# INTRODUCTION

- Phylum Bacteroidetes
- Putative periodontal pathogen.
- Colonizes periodontal pocket and spreads into deeper tissues, including connective tissue and bone
- Isolated from subgingival plaque samples.

# GENERAL CHARACTERISTICS

- Nonmotile
- Gram-negative
- Rod shaped
- Asaccharolytic
- Anaerobic





# COLONY CHARACTERISTICS

- Black colonies on blood agar - (HAFFAJEE AND SOCRANSKY, 1994
- Smooth to rough colony morphotypes (Reynolds et al., 1989).
- Proteolytic activity.
- Only known porphyromonas species isolated from human that produces phenyl acetic acid as a metabolic end product.
- Key test for identification of species include haemagglutination & proteinase activity.

# PREVALANCE

- Presence of P. Gingivalis has also been correlated with periodontal pocket depth (Dahlén et al)
- Healthy cases or sites →low numbers
- Deep periodontal pockets →significantly higher.
- Higher serum titers of antibodies against P.Gingivalis in periodontitis patients than in periodontally healthy have been demonstrated (Naito et al)

# VIRULENCE FACTORS

- (HAFFAJEE AND SOCRANSKY, 1994).

1. Lipopolysaccharides (LPS)
2. Capsular polysaccharide
3. Fimbriae
4. Enzyme activity
5. Outer membrane vesicles
6. Hemagglutinin
7. Protein antigens

# LIPOPOLYSACCHARIDES

- Two distinct lipopolysaccharide macromolecules have been identified in P. Gingivalis strain .
- One of these species contains an anionic polysaccharide with phosphorylated branched mannose repeating units linked to lipid A (A- LPS).
- The other is a polysaccharide with tetrasaccharide repeating units (o-antigen) linked to lipid a (O-LPS) .

# LIPOPOLYSACCHARIDES

- Lipid A is the toxic part of LPS and has endotoxic activity
- Stimulates host inflammatory response indirectly by host derived cytokines (Bartold et al., 1991; Yamaji et al., 1995)
- O-specific antigen and has also significant immunological activity (Takada et al., 1992).
- Kadano et al. Inhibition of the differentiation of rat osteoprogenitor cells into osteoblasts
- Proinflammatory cytokines, including IL-1, IL-6, IL-8 and TNFα,

# CAPSULE

- Polysaccharide heteropolymer on the outer membrane of the bacterial cell.
- Physiochemical barrier for the cell protecting against opsonization and phagocytic host cells. Neutrophils (polymorphonuclear leukocytes) and from desiccation (Chen et al., 1987).
- Antiphagocytic activity

# FIMBRIAE

- Diameter of 5 nm.
- Highly antigenic and show high serum IgA and IgG antibody responses (Ogawa et al., 1990; Yoshimura et al., 1987).
- Binding capacity to host cells including the oral epithelial cells, gingival fibroblasts and endothelial cells, other bacterial species, extracellular matrix protein and salivary proteins (Hamada et al., 1998).
- Mediate the coaggregation of P. Gingivalis and other plaque-forming bacteria such as actinomyces viscosus, streptococcus gordonii and streptococcus mitis
- Induce production of several cytokines from macrophages.(Hamada et al., 2002).

# FIMBRIAE

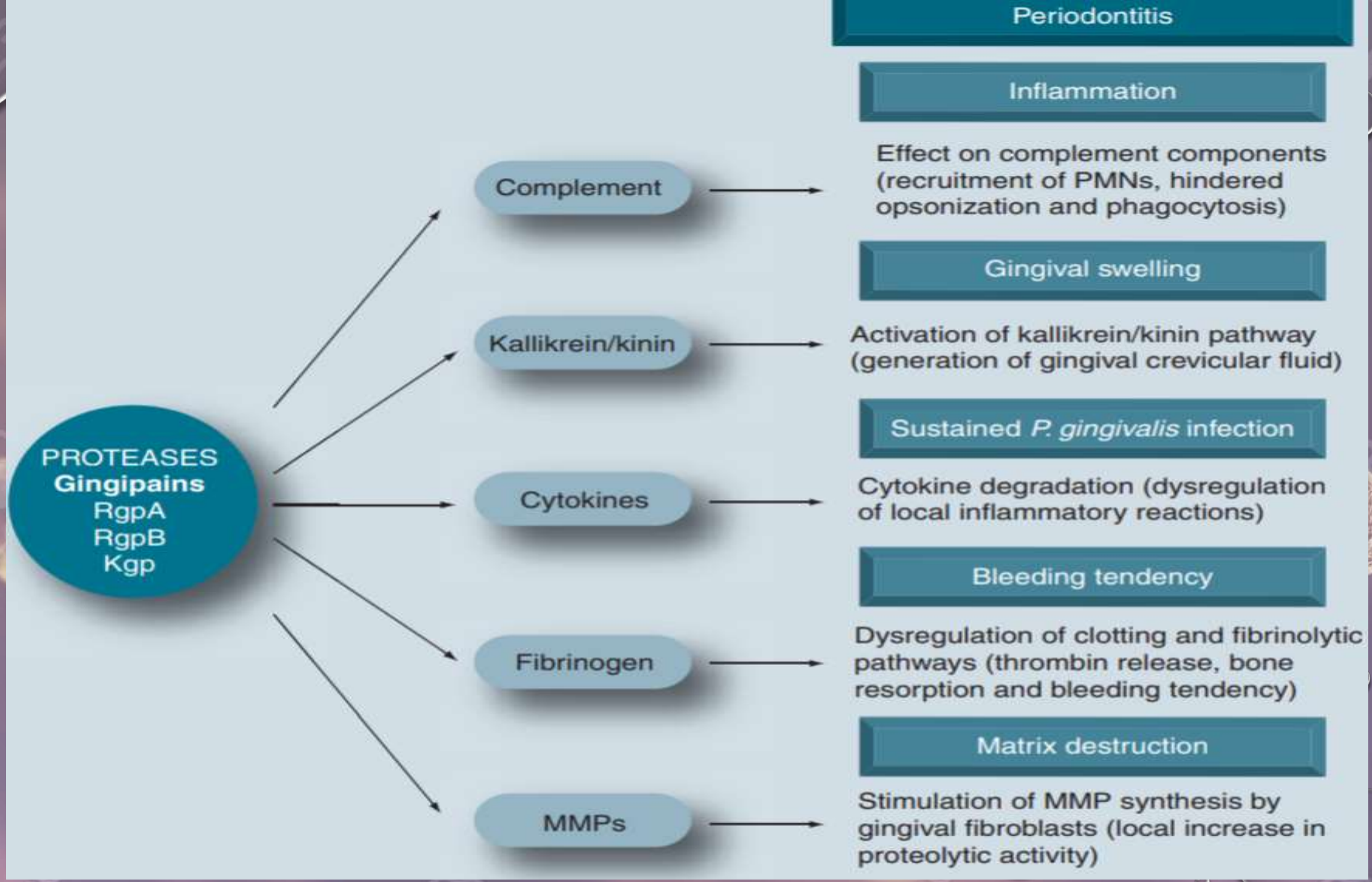
- Major fimbriae:
- Nagakawa et al in 2002 described 6 variants based on the nucleotide sequences as type I, ib, II, III, IV & V.
- Type ii is the most virulent type followed by ib, iv and v.
- Types i and iii are avirulent / noninvasive.
- Minor fimbriae:
- First described by Hamada et al in 1996.

# EXTRACELLULAR PROTEOLYTIC ENZYMES

- The arg-x (R) and lys-x (K) specific extracellular cysteine proteinases → degrade serum proteins including immunoglobulin as well as extracellular matrix proteins.
- This family of cysteine proteinases have been given the name ***"gingipains"*** (Curtis et al., 1999).
- The gingipains constitute a group of cysteine endopeptidases that are responsible for at least 85% of the general proteolytic activity (Potempa et al., 1997) and 100% of the "trypsin-like activity" produced by P.Gingivalis (Potempa et al., 1995).
- Therefore, gingipains are important virulence factors in the periodontal infection.

# MECHANISM OF ACTION OF GINGIPAINS

- Activation of kallikerin/kinin system (HINODE ET AL (1992)
- Activation of blood clotting system
- Degradation of fibrinogen to fibrin
- Disturbances of host defense systems


Periodontitis
Inflammation
Effect on complement components (recruitment of PMNs, hindered opsonization and phagocytosis)
Gingival swelling
Activation of kallikrein/kinin pathway (generation of gingival crevicular fluid)
Sustained P. gingivalis infection
Cytokine degradation (dysregulation of local inflammatory reactions)
Bleeding tendency
Dysregulation of clotting and fibrinolytic pathways (thrombin release, bone resorption and bleeding tendency)
Matrix destruction
Stimulation of MMP synthesis by gingival fibroblasts (local increase in proteolytic activity)
PROTEASES
Gingipains
RgpA
RgpB
Kgp
Complement
Kallikrein/kinin
Cytokines
Fibrinogen
MMPs

# GINGIPAINS

Gingipains as targets for periodontal therapy :

- Vaccines

The first possibility is a vaccination therapy using gingipains for periodontitis. Immunization gingipain R resulted in protection from P.Gingivalis invasion. Antibodies directed against gingipain R are capable of inducing protective immune response against P.Gingivalis infection.

- Inhibitors for gingipains

A proteinase inhibitor reduced porphyromonas gingival growth, suggesting the potential therapeutic effect of gingipain inhibitors in periodontitis.

# PROTEINASES

- A. TRYPSIN LIKE PROTEINASE - GINGIVAIN
- B. COLLAGENOLYTIC PROTEINASE – CLEAVES BASEMENT MEMBRANE – TYPE IV COLLAGEN
- C. OTHER PROTEINASE- DIPEPTIDYLPEPTIDASES

# OUTER MEMBRANE VESICLES

- Gram-negative bacteria form small structures on the outer membrane surface of bacteria named “outer membrane vesicles”.
- This OMV are released from the outer membrane during growth (Handley and Tipler, 1986).
- The OMV of P. Gingivalis may contain several virulence factors including gingipains (Marsh et al., 1989).

# METABOLIC END PRODUCTS

- The bacterial metabolic end-products (Volatile short chain fatty acids, sulfur products and ammonia) can contribute to the *nutritional resources* and support other bacteria within biofilm, as well as toxicity to host cells (Holt et al., 1999)
- The short-chain fatty acids such as ***succinate,isobutyrate*** and ***isovalerate*** can *inhibit the function of neutrophils and T-lymphocyte*
- ***Hydrogen sulfide*** and ***methyl mercaptan*** have been detected in significant phagocytes ,gingival fibroblasts and periodontal ligament cells amounts in periodontal pockets (Persson, 1992).
- ***Ammonia*** is strongly *cytotoxic* to neutrophils and gingival fibroblasts

# COAGGREGATION

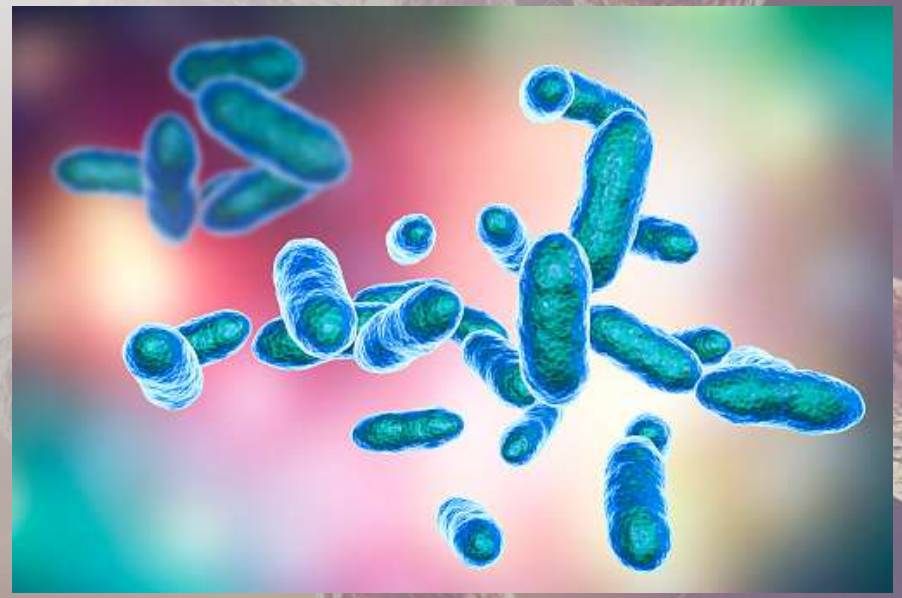
- Highly specific stereochemical interaction of protein and carbohydrate molecules located on the bacterial cell surfaces.
- P.Gingivalis coaggregate with,
- S.Oralis – G3P dehydrogenase of S.Oralis is needed.
- S.Gordonii – 40 kda OMP of P.G is needed
- A.Naeslundi – gingipains of P.G is needed
- F.Nucleatum – needs galactose binding adhesion site.

# TANERELLA FORSYTHIA

# HISTORY

- First isolated at the Forsyth institute from subjects with progressing advanced periodontitis in the mid-1970s
- Described as fusiform bacteroides by Tanner et al.
- Around the same time, T. Forsythia was isolated as one of the bacteroides group from the extensive cultural studies of periodontal infections by Moore and Holdeman at the Anaerobe laboratory of the Virginia polytechnic institute (VPI).
- The species was subsequently detected by culture from oral samples at the Forsyth and VPI laboratories from progressing periodontitis ,endodontic infections , gingivitis and early periodontitis , refractory periodontitis , and peri-implantitis.

# GENERAL CHARACTERISTICS



- Gram –negative
- Obligate anaerobe
- Non-motile
- Spindle-shaped
- Highly pleomorphic rods
- In case of advanced active periodontitis, it is found along with P.Gingivalis.
- It is much higher in subgingival plaque than supragingival plaque.
- Most detected species in refractory periodontitis.

# CULTURE CHARACTERISTICS

- Growth of T.Forsythia is stimulated by n-acetyl-muramic acid
- Shape of T.Forsythia cells and colonies varies depending on the growth
  - Without n-acetyl muramic acid

  a. Large b. Filamentous c. Tapered (fusiform)
  - In presence of n–acetyl- muramic acid or growth-stimulating species

  a. Tiny and opaque b. Pale, pink and speckled circular c. Slightly convex may have depressed centre (donut shape)
  - Also cells become regularly shaped, short ,gram-negative rods.

# CULTURE CHARACTERISTICS

- Antibiotic resistance
- TET(Q), a gene encoding the ribosome protection protein resulting in antibiotic resistance to tetracycline.
- ERM(F) gene codes for erythromycin resistance
- The ability of T. Forsythia to hydrolyze the trypsin like benzyol-dl-arginine-2- naphthylamide, BANA, has been incorporated in the test for periodontal pathogens pioneered by Loesche .

# CULTURE CHARACTERISTICS

- Immunological assays :
- Antibody raised against T. Forsythia cells and used for species identification, did not cross-react with P. Gingivalis, prevotella intermedia.
- They were subsequently used in enzyme-linked immunosorbent assays and immunofluorescence assays molecular identification
- PCR
- DNA probes

# BIOFILM FORMATION

- T. Forsythia can form biofilms in vitro with F. Nucleatum .
- The thickness and structure of T. Forsythia biofilms is influenced by F. Nucleatum.
- Both species co-aggregate when in a planktonic form, and this interspecies binding appears to be critical in the formation and structure of t. Forsythia– F. Nucleatum biofilms
- Favors T. Forsythia growth

# VIRULENCE MECHANISMS OF T.FORSYTHIA:

- Surface components
- Surface-layer associated glycoproteins
- Leucine-rich repeat bspa protein
- Surface lipoproteins glycosidic activity

# SURFACE-LAYER ASSOCIATED GLYCOPROTEINS

- Comprises of two high-molecular-mass glycoproteins of 220 and 210 kda encoded by the TFSA and TFSB genes respectively
- Provides a protective shield.
- Promotes epithelial cell adherence and invasion.
- S-layer proteins are immunogens in periodontitis patients .
- Mediates hemagglutination, which is inhibited by n-acetylglucosamine, and heat denaturation of the proteins.

# LEUCINE-RICH REPEAT BSPA PROTEIN

- BSPA (BACTEROIDES SURFACE PROTEIN A)
- A surface-associated as well as secreted protein
- Leucine- rich repeat family
- Protein–protein interactions that are important in mediating T. Forsythia interactions with the factors and/or components of other bacteria
- Bind to the extracellular matrix component fibronectin and the clotting factor fibrinogen
- BSPA has been shown to trigger the ***release of bone-resorbing proinflammatory cytokines from monocytes***.

# SURFACE LIPOPROTEINS

- Activates host cells to release proinflammatory cytokines
- Induce cellular apoptosis
- Lipoprotein fractions of T.Forsythia – stimulate fibroblasts and monocytic cells to release IL-6 and TNF-α

# GLYCOSIDIC ACTIVITY

- Glycosidases can hydrolyze terminal glycosidic linkages in the complex oligosaccharides and proteoglycans that are abundant in saliva, gingival crevicular fluid and periodontal tissue.
- ***Degradation of oligosaccharides*** and ***proteoglycans*** will affect the *functional integrity of the periodontium* and may promote disease progression.
- The degradation of host oligosaccharides and proteoglycans by these glycosidases can also provide nutrients for other community bacteria.

# TREPONEMA DENTICOLA

- Spirochete
- Motile
- Obligatory anaerobic
- Gram-negative bacteria,
- Account for approximately 50% of the total bacteria present in a periodontal lesion – present in sub gingival plaque.
- T. Denticola increases to large numbers in adult periodontitis but is almost undetectable in oral health (Holt and Ebersole 2005; Sakamoto et al. 2005).
- Associated with acute ulcerative necrotizing gingivitis
- T. Denticola was detected from atherosclerosis lesions

# TREPONEMA DENTICOLA

| VIRULENCE FACTOR | ACTIONS |
|---|---|
| LPS (TLR 4) | Potent stimulator of IL1, PGE2 and TNFα |
| Protease | Degradation of extracellular matrix |
| Dentisilin | Pleiotropic effects. Crucial for the virulence of T.denticola. |
| Major Sheath Protein (MSP) (TLR 2) | Cytotoxic for a wide variety of cells |
| Phospholipase C | Directly or indirectly damages tissue by hydrolysis of membrane phospholipids |
| H2S and Methylmercaptan | Cytotoxic effects that is primarily due to inhibition of cytochrome oxidases. |

# TREPONEMA DENTICOLA

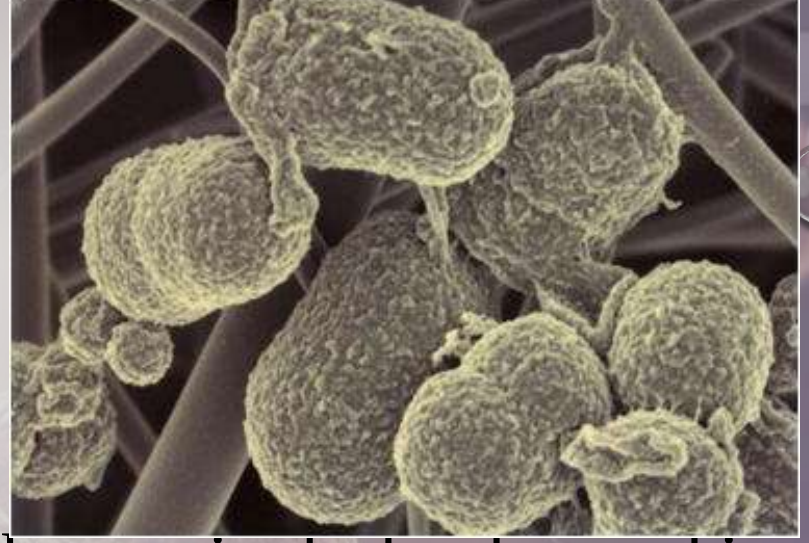
- **<u>DENTISILIN IN TISSUE DESTRUCTION:</u>**
- Induce cytokine production which was induced by cell-surface components, such as peptidoglycan and other components of outer membrane
- ***Peptidoglycan*** isolated from T. Denticola induced the *release of interleukin-1b, interleukin-6, tumor necrosis factor-a , interleukin-8, matrix metalloproteinase-8 and prostaglandins from macrophages.*
- *Monocyte chemoattractant protein-1 and interleukin-8 were also degraded by dentilisin* , which may be associated with evasion of the host defense by T. Denticola.

# TREPONEMA DENTICOLA

**DENTILISIN FOR COAGGREGATION:**

- T. Denticola was co-isolated with P. Gingivalis and T. Forsythia, suggesting that coaggregation and synergy among these species are important in colonization by these microorganisms.
- MSP was reported to be a candidate ligand for coaggregation reactions between t. Denticola and P. Gingivalis or F. Nucleatum
- Dentilsin was also suggested to be involved in coaggregation, long fimbriae (fima) of P. Gingivalis reacted with a 72 kda T. Denticola protein, and this protein was identified as dentilisin

# PREVOTELLA INTERMEDIA

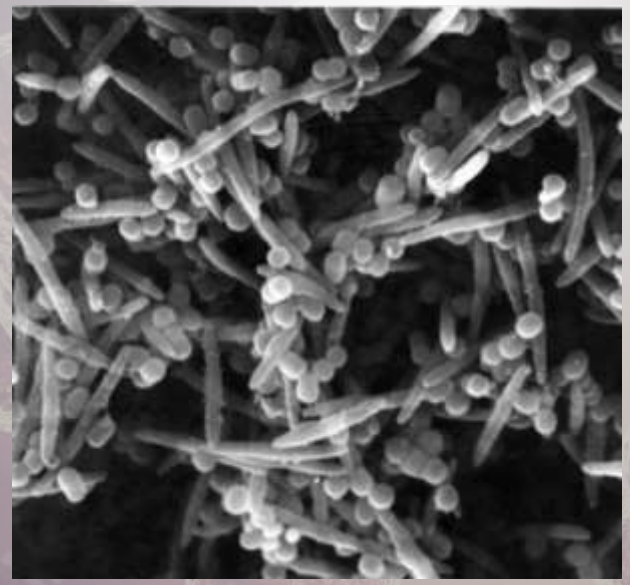


- Gram-negative.
- Short, round-ended anaerobic rod shown to be particularly elevated in ANUG (Loesche et al 1982), and also in certain forms of periodontitis (Herrera et al 2000).
- This species appears to have a number of virulence properties exhibited by p. Gingivalis and were shown to induce mixed infections.(Hafstrom & Dahlen 1997).
- It has also been shown to invade oral epithelial cells in vitro (Dorn et al 1998).

# PREVOTELLA INTERMEDIA

| VIRULENCE FACTORS | ACTIONS |
| --- | --- |
| • Proteases | Degrade matrix components, host cell receptors and Ig's |
| • Hemolysins | Lysis of RBC's |
| • LPS | Potent stimulator of IL1, IL6 and IL8 release.<br>Causes periodontal tissue destruction and alveolar bone resorption. |

# FUSOBACTERIUM NUCLEATUM



- Gram-negative
- Anaerobic
- Spindle shaped (cigar shaped) rod that has been recognized as part of the subgingival microbiota for over 100 years. (Plaut 1894, Vincent 1899).
- Most common isolate found in cultural studies of subgingival plaque samples comprising app. 7-10% of total isolates. (Moore et al 1985).

# FUSOBACTERIUM NUCLEATUM

- ROLE IN PATHOGENESIS
- Increased secretion of IL-8 (Han et al 2000).
- Induce apoptotic cell death in mononuclear and polymorphonuclear cells (Jewett et al 2001).
- Cytokine, elastase and oxygen radical release from leukocytes (Sheikhi et al 2000).
- Bridging organism.

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thank you