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We are all in the gutter but some of us are looking at the stars - A Review on Aggregatibacter Actino my cetemcomitans

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Abstract

Aggregatibacter actinomycetemcomitans is a gram-negative bacterium that is present in the oral cavity of a large population. The prevalence of this bacterium shows a great variation depending on geographic origin, age and lifestyle.

A. Actino my cetemcomitans found in a normal flora in many healthy individuals, but is a major causative agent in stage III and IV periodontitis. Periodontitis is a chronic inflammatory condition of the periodontal tissues, which leads to periodontal attachment loss and destruction of the alveolar bone that houses the teeth. It is of considerable interest to know that A. Actino my cetemcomitans possesses so many virulence factors but unfortunate that only few have been studied. In the hope to understand and eradicate this pathogen, it is crucial that in- depth understanding of its genetic origin, regulation and mechanism of action of these factors as well as the recent anti-virulence strategies is briefly elaborated.

Keywords: Aggregatibacter Actino my cetemcomitans, Leukotoxin, Periodontitis, Virulence factors, Anti-virulence therapy.

Introduction

One of the major challenges is to understand how the vast diversity of bacteria can thrive with human

organisms, & what mutual signalling pathways exist to allow the interaction between the prokaryotic world and the eukaryotic world within the host organism and also cause disease. Periodontitis is one such bacterial induced chronic inflammatory disease, associated with loss of the supporting connective tissue and alveolar bone around teeth.

Aggregatibacter Actino my cetemcomitans is a gram negative, anaerobic, facultative bacterium, that has been described as a member of the indigenous oral microbiota of humans associated with the causation of periodontitis.

Virulence is the degree of pathogenicity or ability of the organism to cause disease measured by experimental procedure. The function of Virulence factors is to either harm the host or inhibit the growth of other bacterium or act as a growth factor for the bacteria itself. Aggregatibacter Actino my cetemcomitans expresses several virulence factors, which activates a host response that could be associated to the pathogenesis of periodontitis. The "crown jewel" of the virulence factors of A. Actino my cetemcomitans has long been its leukotoxin.[1] However, a cytolethal distending toxin (CDT) has also been identified, making this species the only member of the oral microbiome to produce these two, or any of the two protein exotoxins.[2]

The immunological responses elicited by the host to its lipopolysaccharide can be used in classifying (serotyping) the virulence identity of each one of its strains. A. Actino my cetemcomitans is also equipped with a wealth of outer membrane vesicles and cytokine-binding molecules. Increased knowledge about bacterial virulence markers in periodontal disease may be important tools in future strategies for personalized dentistry.

Currently seven serotypes of this bacterium (a-g) are recognized based on the O-polysaccharide of the lipopolysaccharide (LPS). The virulence potential of A. Actino my cetemcomitans appears to vary among strains, and specific serotypes/clonal types of the bacterium have been reported to be more prevalent in individuals with aggressive forms of the disease.

Targeting "pathogens" will not necessarily cure disease, since other organisms with similar activities might take their place. Therefore, it may be fruitful to focus on the specific virulence factors that contribute to disease, rather than on the microorganisms that produce those factors.[3]

This article throws light upon the virulence mechanisms and factors of A. Actino my cetemcomitans with a brief insight of ant virulence therapies for the same.

Aggregatibacter actinomycetemcomitans

Killian & Schiott were first to demonstrate that A. Actino my cetemcomitans was present in dental plaque. A Actino my cetemcomitans is a gram negative, facultatively anaerobic that is non-motile, non-sporing, capnophilic, fimbriated small rod of 0.4–0.5 µm x 1.0–1.5 µm in size. Microscopically, the cells may appear coccobacillary. It is non-haemolytic, and oxidase and catalase positive.[4]

The colonial morphology is similar to the internal star-shaped or crossed cigar morphology form embedding in the agar that gives A. Actino my cetemcomitans its name. It forms small, rough surface, translucent colonies, with internal star shaped morphology. Smooth colonies are also present that lack star shaped morphology and are less virulent.

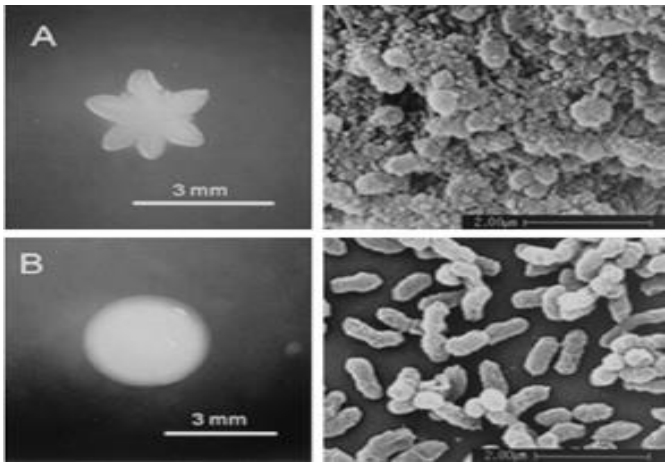

Figure 1:

Morphology of A. Actino my cetemcomitans strain D7S and its smooth-colony mutant. Left side, colonies on sTSB agar; right side, scanning electron micrographs of bacteria on the coverslip surface. (A) D7S; (B) D7S-smooth**.** (Ying Wang et al, 2002)

This bacterium has a complex life cycle, it colonizes the oral mucosa early in life and is inherited by vertical transmission from close relatives. It moves from initial colonization site to gingival crevice and competes with bacteria. When A. Actino my cetemcomitans is allowed to stay, proliferate, and express virulence factors, it has the potential to initiate periodontal diseases.[5]

Surface ultrastructure of A. Actino my cetemcomitans Fimbriae

Actinomycetemcomitans fimbriae occur in bundled peritrichous arrays, 2 pm in length and 5 nm in diameter. Fimbriated strains produce star positive colonies; non-fimbriated strains are designated star negative. They help in adhesion. However, non-fimbriated A. Actino my cetemcomitans also function in adhesion.[6]

Vesicles

A. Actino my cetemcomitans vesicles (blebs) are lipopolysaccharide in nature, originate from and are continuous with the outer membrane.[7] Vesicles exhibit leukotoxic activity.

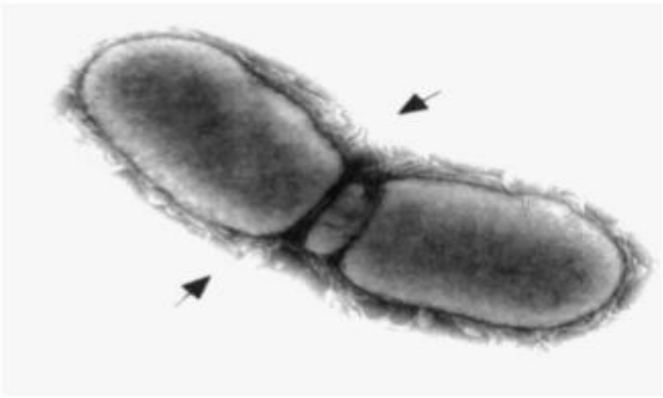

Figure 2:

Scanning electron micrograph of A. Actino my cetemcomitans strain SUNY 465 revealing the presence of large amounts of fibrillar membranous vesicles (arrows) on the cell surface. (Meyer & Fives- Taylor, 1999)

Extracellular amorphous material

The material is a glycoprotein, exhibiting both bone-resorbing activity and adhesive properties. Furthermore, A. Actino my cetemcomitans strains, which normally exhibit low levels of adhesion, exhibit increased levels of adhesion when suspended in extracellular amorphous material, a phenomenon termed conveyed adhesion.[8]

Virulence of A. actinomycetemcomitans

Twenty years ago bacterial virulence was equated to the production of toxins. However, over the past few years, bacteria- host interaction is also considered a virulence mechanism. Adhesion to cells and invasion of cells are two key virulence activities of bacteria. Another important mechanism contributing to bacterial virulence is the ability to evade host defence mechanisms.

Virulence mechanisms of A. actinomycetemcomitans

Iron Acquisition

Iron is essential for bacterial growth. Under iron-limiting conditions, there is increased transcription & translation of all biofilm determinants like fimbriae, lipopo Ly saccharide, & extracellular polymeric substances. A. Actino my cetemcomitans has a lipopolysaccharide that

binds to haemoglobin and could play a role in iron acquisition. Studies have shown that A. Actino my cetemcomitans binding to haemoglobin depends on at least two cell surface proteins with molecular masses of 40 & 65 kDa.[9] Due to the presence of a non-functional tbpa gene encoding transfer binding protein, it has shown that all strains tested bound to lactoferrin, haemoglobin, & haemin but not transferrin.

Adherence of A. actinomycetemcomitans

If a bacterium cannot adhere to a particular environment it cannot survive, thus regarded as an important virulence mechanism. In addition to adhesion to the host, bacterial adhesins can also be used to bind to bacteria of either same or different species.[10]

A novel operon termed tight adherence (tad) operon, comprising seven adjacent genes (tag A-6) was identified. Tad negative bacteria lacked the long-bundled fibrils found in auto aggregating A. Actino my cetemcomitans. The flp-1 gene lies upstream of the tad operon, & inactivation of the gene resulted in failure to produce fibrils and loss of adherence. Mutations of flp and tad genes resulted in no evidence of colonization or bone loss in an oral colonization model demonstrating that flp gene cluster is important for virulence of this bacterium.[11]

Fibronectin-binding proteins an 11 kDa protein that has been named Com $E_{1,}$ binds to unique site of fibronectin - Fn $III_{9\text{-}10}$ domain and is crucial for binding to and invasion of cells. [12] Aae, an autotransporter protein which is a homologue of Hap (Haemophilus auto transporter protein) in H. influenza. Inactivation of this in two strains of A. Actino my cetemcomitans (SUNY 465 & ATCC 29523) revealed 70% reduction in epithelial cell binding.[13]

Mintz identified a gene encoding protein EmaA (extra-cellular matrix protein adhesin). It has been found that it binds to type 1 collagen.[12] In addition, poly - N-acetyl-glucosamine, which mediates intercellular adhesion & attachment of cells to abiotic surfaces helps in formation of biofilms.

Invasion of A. actinomycetemcomitans

Bacteria are protected from immune defences & from antibiotics when they are within cells. A. Actino my cetemcomitans was the first invasive period on to pathogenic bacteria to be reported. It was found that A. Actino my cetemcomitans invaded KB cells through cytochalasin D sensitive mechanisms, implicating the actin cytoskeleton invasion of this organism. Interestingly smooth colony variants of A. Actino my cetemcomitans invaded more efficiently than rough variants.[14]

Invasion involves the formation of cell surface craters or apertures with lip like rims, with bacteria appearing in the host cell cytoplasm within 30min.[15] Another mechanism is by interacting with the microtubules, thus preventing the egress of AA from host cells. A. Actino my cetemcomitans possess phosphorylcholine on the cell surface, taken up by Vascular endothelial cells which may allow A. Actino my cetemcomitans to enter the bloodstream.[16]

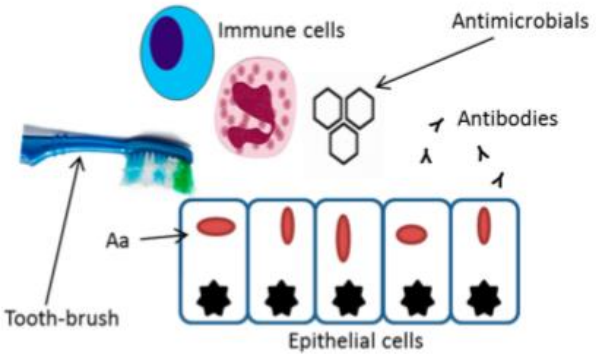

Figure 3:

Invasion of A. Actino my cetemcomitans into epithelial cells (Jan Oscar's son et al, 2019)

Toxins of A. actinomycetemcomitans

Classically, the bacterial toxins are divided into exotoxins and endotoxins. while endotoxins are membrane compounds of gram-negative bacteria which act locally and at a A. Actino my cetemcomitans is the only oral bacterium to produce two exotoxins. These toxins are the leukotoxin and a version of cytolethal distending toxin. A third potential toxin is a homologue of H. pylori CagE

Leukotoxin

The A. Actino my cetemcomitans leukotoxin (LtxA) is an example of an RTX toxin. It is a secreted protein of 114kDa. The leukotoxin gene (LtxA) resides in an operon consisting of four genes, C, A, B and D.[17]

A. Leukotoxin Production and Secretion

The operon consists of four coding genes and an upstream promoter region.[18] The gene LtxA encodes the LtxA protein, ltxC a protein required for the posttranslational acylation of LtxA, and ltxB and ltxD proteins needed for the transport of the LtxA to the bacterial outer membrane. For regulation of the LtxA expression, there is a promoter region located upstream of the ltxC gene, and genetic differences within this region result in different genotypes with various LtxA expression.

The JP2 genotype was first identified by Brogan and co-workers and was a serotype b isolate with a 530-base pair (bp) deletion in the ltx CABD promoter. An insertion of 886 bp, and a 640 bp deletion in the ltx promoter, has been discovered. These three different genotypes are all associated with high virulence due to enhanced production of LtxA. The secretion of LtxA is mediated by a Type I secretion system and associated with the bacterial outer membrane not found in other RTX proteins.[19]

B. Leukotoxin pathogenesis

ltxA shows high target cell specificity and affects only cells of hematopoietic origin from humans and some other primates, mainly the different leukocyte populations.[20] Ltx A mainly binds to Lymphocyte function– associated antigen 1 (LFA-1) receptor.[21] Taken together, several of the mechanisms by which LtxA affects the cells are also involved in the pathogenic mechanisms of many inflammatory disorders, such as periodontitis.

Interaction of LtxA with White Blood Cells

In a study by Lally et al., they determined that amino acid residues 688–941 containing a non a peptide glycine-rich repeats are necessary for LtxA to kill target human cells and critical for cytotoxicity.[22] LtxA contains 12 such repeats which are essential for the unique species recognition of LtxA. It interacts with the host cell membrane and its receptors, whose biology of interaction is further explained below.

Interactions with Target Cell Membranes

Initially before interacting with LFA-1 receptor, LtxA may associate with the host cell plasma membrane and induce changes. The earliest observable effects of LtxA on target cells is an increase in cytosolic (Ca2+), followed by a decrease in membrane potential.[23]

LtxA-treated cells demonstrated two membrane defects—the collapse of the microvilli normally present on the outer surface of cells, and the formation of cell surface depressions, followed by lipid-lined cavities. Membrane is not disrupted by pore formation, but rather by membrane destabilization, manner independent of LFA-1.

Additionaly, LtxA is known to almost irreversibly bind to cholesterol. It contains two cholesterol recognition /amino acid consensus (CRAC) sites—CRAC336 which is highly conserved among RTX toxins, and CRAC503

which is unique to LtxA. LtxA requires cholesterol for cytotoxicity. [24]

Interaction of LtxA with its receptors

The lymphocyte function-associated antigen-1 (LFA-1) integrin is the functional receptor for LtxA. However, a number of recent studies have identified additional receptors that might be involved in LtxA recognition.

Lymphocyte function-associated antigen-1 (LFA-1):

LFA-1 β2 Intergrin is a heterodimer on myeloid and lymphoid cells. It is a key adhesion molecule that aids in leukocyte migration via interaction with the intercellular adhesion molecules (ICAMs) expressed on vascular endothelial cells. [25] It is composed of two non-covalently associated subunits: CD11a (αL) and CD18 (β2). The smaller CD18 subunit (85 kDa), contains three metal-ion binding sites.

P2 Receptors

P2 receptors neutralize nucleotides which if released into extra cellular environment can lead to cell death. Munksgaard and colleagues hypothesized that like the E. coli α-HlyA, LtxA interacts with P2X receptors in an ATP-dependent manner to mediate lysis. However, additional experimental controls must be conducted before definitive connections can be made between P2X receptors and LtxA.[26]

Fas receptor

DiFranco and colleagues recently demonstrated that LtxA kills lymphoma cells in a caspase-8-dependent manner and found that Fas receptor to be an essential component in the LtxA-mediated killing of these cells.[27]

Host cells targeted by leukotoxin of A. Actino my cetemcomitans

a. Monocytes and macrophages

Monocytes are the most sensitive to LtxA and the kinetics of this process are very rapid. LtxA has been shown to preferentially target CD14+ monocytes that express the purinergic receptor, P2X7R. LtxA/ LFA-1 interaction leads to the activation of caspases. The LtxA is internalized in an LFA-1/B2 integrin dependent manner where LtxA is shuttled to the lysosome. It ruptures the lysosomal membrane, allowing leakage of lysosomal proteases, such as cathepsin D, into the cytosol leading to rapid and irreversible cell death.[28]

b. polymorphonuclear leukocytes

PMNs treated with LtxA demonstrated signs of granule translocation towards the cell surface, the formation and shedding of cell membrane blebs and karyorrhexis. PMNs rapidly release the granule components resist in, MMPs, myeloperoxidase, lysozyme B glucuronidase, lactoferrin, neutrophil elastase and other lysosomal enzymes into the extracellular compartment.[29]

Several studies proposed that neutrophils exposed to LxtA can activate and release neutrophil extracellular traps (NETs) in a process called NETosis. This is a slow process and can proceed to cell death in the presence of LtxA.[29]

c. lymphocytes

Lymphocytes were thought to be resistant to LtxA-mediated cytotoxicity. However, LtxA-induced cell death in lymphocytes occurs much slower than cell death in cells of myeloid origin.[30] Studies have identified a critical role for the Fas (CD95) death receptor in LtxA-mediated cell death in T-lymphocytes by a caspase-dependent mechanism. Classical features of apoptosis were observed.[31]

d. Other host cell types targeted by leukotoxin A

Erythrocytes

Certain strains of A. Actino my cetemcomitans produce a hemolytic protein. An LtxA mutant strain was not hemolytic, and purified LtxA exhibited dose-dependent lytic behaviour against both sheep and human

erythrocytes, suggesting that LtxA possesses some hemolytic activity.[32]

Endothelial cells

Diet Mann and colleagues investigated the effect of LtxA on human microvascular endothelial cells. They found that high doses of LtxA decreased cell proliferation by causing cell cycle arrest in the G2/M phase and induced apoptosis leading to destruction of the gingival tissue, inhibiting the barrier function of the tissue and therefore promoting invasion of A. actinomycetemcomitans.[33]

The primary target of LtxA is human immune cells. Reported effects in erythrocytes and fibroblasts require significantly higher toxin concentrations and much longer incubation times than the observed effects in human lymphocytes.

What is the role of LtxA in the pathogenesis of periodontitis?

The bacterium induces pro-inflammatory factors and tissue damaging agents, inhibits the killing actions of the key antibacterial components of immunity (phagocytes) and protects the bacteria from immune mediated killing.[34] IL-1B that is secreted act as the major activator of bone resorption by promoting osteoclast survival and activation.

Macrophages play a significant role in periodontal tissue remodelling, & their ability to secrete large amounts of IL-1 indicates their involvement in the enhanced bone loss seen in periodontitis. The ltxA induced interaction with accumulated macrophages in the periodontal tissues play a crucial role in the pathogenic potential of A. actinomycetemcomitans infection.

Cytolethal distending toxin

The CDT family comprises a number of bacterial protein exotoxins that is expressed by several Gram-negative species. The production of CDT by A. Actino my cetemcomitans was reported by Sugai et al.[35] and it is the only known oral species with this property.

The CDT holotoxin consists of subunits CdtA, CdtB, and CdtC. While CdtA and CdtC subunits mediate the internalization of the CdtB into the cell, the latter is translocated to the nucleus, causing its deleterious effects on the host cells. This subunit is functionally homologous to deoxyribonuclease I, hence it can cause DNA damage.[36]

An estimated 66% to 86% of its strains express a CDT. It causes DNA damage, cell cycle arrest, and eventually apoptosis to the intoxicated cells. This has been shown in structural cells, denoting that it can compromise the structural integrity and homeostatic capacity of the tissues.[37] CDT may also subvert the phagocytic capacity of macrophages and subvert their cytokine producing capacity therefore affects the local immunity.[38]

CDT stimulates pro-inflammatory and osteolytic cytokine production by the intoxicated host cells mainly receptor activator of nuclear factor kappa-B ligand (RANKL) therefore potentiates bone destruction. Additionally, CDT acts directly on pre-osteoclasts, it may also induce apoptosis and hinder their differentiation to osteoclastic cells, thereby contributing a disbalanced bone remodelling equilibrium that leads to periodontal breakdown. [39]

CagE

H. pylori is well known for its possession of the Cag pathogenicity island, with associated cytotoxin-associated genes A and E (cagA ⁄ E) which may contribute to the gastric cancer associated with infection by H. pylori. Teng & Hu using expression cloning, have identified a CagE homologue in A. Actino my cetemcomitans which may lead to its possible association of A. Actino my cetemcomitans with oral dysplasia and oral cancer.[40]

Lipopolysaccharide (LPS)

Like other Gram - negative species, A. Actino my cetemcomitans surface is covered by lipopolysaccharide (LPS), a potent proinflammatory molecule. It comprises of a group of structurally related molecules in which the O-specific polysaccharide chain (O-antigen), formed by oligosaccharide repeating units, is the most variable portion.

Structure of LPS

LPS comprises a lipid A component, core oligosaccharides and an O- antigen. The O-specific polysaccharide chain (O-antigen) is the most variable portion in the LPS. The O-antigen consists of a large variety of sugar residues in many combinations and glycosidic linkages. Serotypes from a to f, as well as to non-serotypeable, is based on the structural differences in the O-antigen part of LPS.[41]

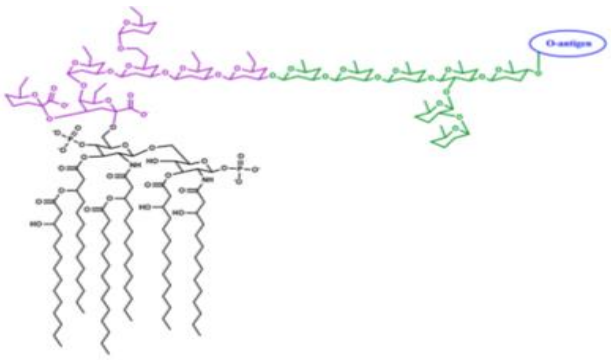

Figure 4:

Structure of LPS (Georgios N. Belibasakis et al, 2019)

Effect of A. Actino my cetemcomitans lipo polysaccharide on epithelial cells

Human epithelial cells have been shown to respond to *A.* Actino my cetemcomitans LPS by expressing IL-15 which results in enhanced IFN-γ production and proliferation of human T cells. Moreover, A. Actino my cetemcomitans LPS causes widening of the intercellular spaces in primary tissue cultures mimicking the JE, a phenomenon not observed with Porphyromonas gingival is LPS.[42]

A. Actino my cetemcomitans LPS is able to enhance the phagocytosis of collagen by fibroblasts resulting in imbalance in regeneration of the gingival tissue. It also stimulates the production of IL-6 and IL-8, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor 2 (PAI-2) by human gingival fibroblasts.[43]

Effect of A. Actino my cetemcomitans lipo poly saccharide on immune cells

A. Actino my cetemcomitans LPS enhances the IL-12 production by m Dendritic Cells leading to stimulation of IFN-γ expression of natural killer cells and undetectable levels of IL-4, which together may cause the polarization of naïve T cells toward the Th1 type response.[44]

Neutrophils produce reactive oxygen species (ROS) when responding to whole bacteria or their components. A. Actino my cetemcomitans LPS has been shown to be more potent inducer of neutrophil ROS production than, for example, P. gingival is or Prevotella intermedia LPS. Moreover, A. Actino my cetemcomitans LPS stimulate the production of inflammatory cytokines IL-1β and TNF-α by PMN more efficiently than P. gingival is LPS.[45]

Effect of A. actinomycetemcomitans lipopolysaccharide on osteoblasts

A. actinomycetemcomitans LPS has capacity to increase the inducible nitric oxide synthase (iNOS) activity and induce the nitric oxide (NO) production by human osteoblast-like cell line. If osteoblasts produce NO rapidly, when responding to bacterial infection, it may lead to bone resorption.[46]

Outer membrane vesicles (OMVs)

Outer Membrane Vesicles (OMVs) of Gram-negative bacteria are spherical membrane-enclosed nanostructures

that are released from the outer membrane. Biologically active virulence factors such as CDT and OmpA can be transported into HeLa cells and human gingival fibroblasts via A. actinomycetemcomitans OMVs.[47] OMVs are also involved in the export of leukotoxin, peptidoglycan-associated lipoprotein (Pal), and the chaperonin GroEL to host cells.[48] OMV proteome also exhibits multiple offensive and defensive functions, such as drug targeting, iron acquisition, and immune evasion.

A role of A. Actino my cetemcomitans OMVs in serum resistance can be hypothesized based on observations that the vesicles could bind to the complement system regulator C4-binding protein in an OmpA-dependent manner. Evidence that A. Actino my cetemcomitans OMVs carry NOD1- and NOD2-active peptidoglycan, and upon vesicle internalization into non-phagocytic human cells such as gingival fibroblasts, the OMVs can act as an innate immunity trigger.[49]

Fc-binding proteins

Fc binding proteins have been associated with A. Actino my cetemcomitans. A heat-modifiable membrane protein of A. Actino my cetemcomitans shown to bind to both murine and human antibodies. The gene encoding this protein was cloned and encoded a protein of 35 kDa, which was termed omp34.

Tolo & He gland demonstrated that the Fc- binding proteins inhibits the ability of opsonizing antibodies to bind polymorphonuclear leukocytes and reduces phagocytosis by 90%. They also would inhibit complement activation.[50]

Bacteriocins

Actino bacillin is a bacteriocin that is active against S. sanguis, Streptococcus uberis and A. viscous. Actino bacillin is associated with both the bacterial cell surface and extracellular vesicles. It increase the permeability of the cell membranes of target bacteria, which leads to leakage of DNA, RNA and macromolecules essential for growth.[51] Lima FL et al is olared a bacteriocin named as Actino my cetemcomitin from A. Actino my cetemcomitans P (7-20) strain that is active against Pepto streptococcus anaerobius ATCC 27337.

Cytotoxins

Many oral bacteria express toxins that inhibit human fibroblast proliferation, but the heat-labile cytotoxin produced by A. Actino- my cetemcomitans is especially cytotoxic. The toxin is considered a virulence factor due to its impact on fibroblast viability. One toxin identified as a 50-kDa protein inhibits DNA synthesis in the fibroblast.[52] A. Actino my cetemcomitans surface-associated material at very low concentrations has also been shown to inhibit fibroblast proliferation. The active component of surface-associated material, designated Gap stein, is an 8-kDa protein. It inhibits cells in the G2 phase of the cell cycle.[53]

Cytokine inducer proteins secreted by bacterium

a. Heat shock proteins

Several authors have reported in A. Actino my cetemcomitans the presence of HSPs including GroEL-like (HSP-60) and DnaK like (HSP - 70) proteins. Protein homologous to GroEL-like HSP found in the surface associated material of A. Actino my cetemcomitans has osteolytic activity by murine bone resorption assay. Purified native GroEL - like HSP from A. Actino my cetemcomitans promotes epithelial cell proliferation at lower HSP concentrations, but has a toxic effect on epithelial cells at higher HSP concentrations.

b. Cell stress protein, chaperonin 60

Potent bone degrading molecule. This molecular chaperone, which is normally intracellular, appears to be secreted by this bacterium and stimulates bone resorption by acting as an osteoclast "growth factor".

Future directions

Anti-Virulence therapies

Understanding the functions, mechanisms of action and target cells of the virulence factors of an organism is essential in order to develop antimicrobial strategies and formulate therapeutic agents specific for the disease-causing virulence factor of the organism. Antibiotic resistance is increasingly becoming more prevalent.[54] This necessitates the exploration of other approaches to combat bacterial infection. LtxA represents an ideal anti-virulence target, as it is more prevalent in disease-associated strains of A. Actino my cetemcomitans.[55]

In a study conducted by Karin H. Ishikawa, they suggested the use of lactobacilli post biotics to reduce biofilm formation and alter transcription of virulence genes of A. Actinomycetemcomitans. Lactobacilli postbiotics may impair colonization/ virulence of A. Actino my cetemcomitans. The effect of the postbiotics of lactobacillus acidophilus La5 on A. Actino my cetemcomitans, including its ability to partially inhibit biofilm formation and interfere in mature biofilm formation, & attenuation of expression of major virulence factors, suggests its potential to impair A. Actinomycetemcomitans colonization. However further studies are needed to evaluate its effects as some species of lactobacilli were not successful in the same.[56]

In another study by En Hyang Chang and Angela C. Brown proposed that epigallocatechin gallate found in green tea leaves, have strong inhibitory activity against the LtxA produced by A. Actinomycetemcomitans. Satio et.al demonstrated that catechins, including EGCg inhibits the activity of outer membrane vesicles associated LtxA. However, they also demonstrated that the molecule increases the amount of LtxA produced, which raises important concerns about its possible therapeutic use. [57]

Maryam Pourhajbager & Abbas Bhador conducted a study focusing on the attenuation of A. Actino my cetemcomitans virulence using curcumin- decorated nano phytosomes- mediated photo- Sono- antimicrobial chemotherapy. They concluded that cur- Nphs- PSACT could reduce the cell viability, metabolic activity and biofilm growth in A. Actinomycetemcomitans by downregulating the expression of rcpA, qseB, qseC genes.[58]

Leukotoxin as a potential therapeutic agent

Similar to BOTOX and ONTAK, LtxA is being investigated as an therapeutic agent of haematological malignancies and immune mediated diseases. LFA-1 and other B2 integrins, are known to be over expressed on WBCs in leukemias, lymphomas, auto immune diseases and inflammatory diseases. Additionally, LFA-1 is known to mediate migration of auto reactive immune cells to various organs in auto immune diseases, such as thyroiditis, psoriasis, multiple sclerosis, and rheumatoid arthritis. Because LtxA specifically targets the active conformation of LFA-1, LtxA may represent as a novel targeted biotherapy for WBC disorders and is being developed as such.[59,60]

Conclusion

A. Actinomycetemcomitans has the capacity to employ a plethora of virulence mechanisms closely associated to the pathogenesis of periodontitis. It produces various toxins some of which are unique only to A. Actino my cetemcomitans. Not only the toxins but other mechanisms such as iron acquisition, invasion and adhesion also contribute to the virulent activities of the organism. Without doubt, individuals carrying A. Actinomycetemcomitans genotypes with a proven enhanced leukotoxin production have significantly increased risk to develop disease.

The important role of LtxA in A. Actino my cetemcomitans pathogenicity has made the toxin an attractive target for scientific study on anti-virulence strategies, which have potential use as antibiotic-free therapeutics. This can overcome the problem of antibiotic resistance prevalent in the periodontal pathogenic organisms. Understanding the virulence properties goes a long way in the development of various diagnostic and therapeutic agents.

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