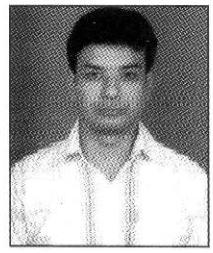
ACTINOBACILLUS (AGGREGATIBACTER) ACTIONOMYCETEMCOMITANS : A PUTATIVE PATHOGEN IN PERIODONTAL DISEASE



Authors
Dr Vikas Verma
B.D.S. , M.D.S. (K.G.M.U., Lucknow)
Sr. Lecturer
Dr Amit Goel
B.D.S. , M.D.S. (K.D.C.R.C.,Moradabad)
Sr. Lecturer
Teerthanker Mahaveer Dental College & Research Center, Moradabad (U.P.)

Introduction

The periodontal diseases affect the gums (gingivae), the periodontal ligament and the alveolar bone. Inflammation of the gums affects all of us at some time in our lives, and is known as gingivitis. If, in addition to the inflammation in the gums, the periodontal ligament and alveolar bone that support our teeth are also affected, and begin to be destroyed, the disease is known as periodontitis. This term encompasses a number of clinically defined conditions. The periodontal diseases are not classical exogenous infectious diseases, and there is good evidence that they are caused by certain members of the normal oral microbiota, principally gram-negative anaerobes. The periodontal disease with the clearest association with an oral bacterium is a curious condition known as localised aggressive periodontitis (LAP), previously known as localised juvenile periodontitis. This disease affects only certain teeth (incisors and premolars) and causes rapid loss of the alveolar bone of the jaw leading to tooth loss. The major causative agent of LAP is Actinobacillus actinomycetemcomitans.

The genus Actinobacillus includes species isolated from humans and mammals. The only species routinely isolated from the oral cavity is Actinobacillus actinomycetemcomitans, so named because it is frequently isolated with Actinimyces spp. from actinomycotic lesions. The reason for this association is unknown. Multiple biotypes and five serotypes (a-e) have been described. This species is a major infectiye agent in particularly aggressive forms of periodontal disease in adolescents (localized aggressive periodontitis) and rapidly destructive periodontal disease in adults.

History

A.a is a member of the genus Actinobacillus that belongs the family pasturallaceae.

A.a was first isolated from a cerivcofacial actinomyceties lesion in 1912 by Klinger who designated it as bacterium actinomycetemcomitans.

This name was changed twice first by Lieske in 1921 to bacterium comitans and later by Topley and Wilson in 1929 to Actinobacillus actinomycetes comitans .

Term Actinobacillus refers to the star shaped inner structure seen in colonies on selective agar medium and to the short rod like or bacillary nature of the cells.

The name actinomyecetemcomitans arises from core refers to its close association with actinomyces israelli in actinomycetem lesion.

Habitat and Transmission

Primary habitat is unknown, but is likely to be subgingiyal sites of humans and mammals. Infection is endogenous.

Structure

Structure : A.a is a gram negative, non-spore forming, non-motile, capnophilic, facultative anaerobic coccobacillus.

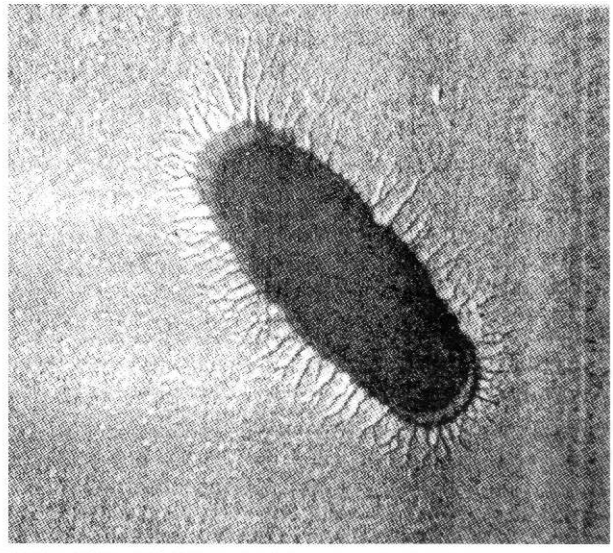
The cells of *A.a* is small, short (0.4-1μm), straight or curved rods with rounded ends.

Electron microscopic shows bleb like structure on cell surface.

A.a can be classified into six serotypes- a, b, c, d, e, f

Based on surface polysaccharides located on the O side chains of lipopolysaccharides. Serotype b is associated with aggressive periodontitis.

A. Actinomycetemcomitans



Culture and identification

Grows as white, translucent, smooth, non-harmolytic colonies on blood agar.

Grows best aerobically with 5-10% carbon dioxide.

Selective media available for identification- tryptone-say-serum-bacitracin-vancomycin agar yields white, translucent colonies with a star shaped internal structure.

Identified also by sugar fermentation and assimilation reactions and acid end products of carbohydrate metabolism.

MECHANISMS

Adhesion

A. actinomycetemcomitans adhesion to the gingival crevice epithelium is likely a key step in its colonization and in the subsequent tissue destruction associated with periodontal disease. Adhesion of A. actinomycetemcomitans to the tooth surface is also important and is more efficient in fimbriated strains. Unlike many later colonizers, A. actinomycetemcomitans has few coaggregation partners and coadhesion has only been demonstrated with F. nucleatum.

Nonspecific adhesion

Certain strains, especially fresh clinical isolates, generate tenacious biofilms on solid surfaces, such as plastic, glass, and hydroxyapatite. This nonspecific adherence requires the tad locus, a cluster of seven genes (tadABCDEFG) associated with the formation of long fibrils and bundled pili. This formation of strongly adherent biofilms could be an early step in A. actinomycetemcomitans colonization of the tooth surface.

Specific adhesion to epithelial cells

Most A. actinomycetemcomitans strains that have been tested to date adhere to epithelial cells strongly. Cell surface entities that mediate adherence include fimbriae, Ex AmMat, and vesicles. However, A. actinomycetemcomitans strains that express the smooth colonial phenotype bind strongly to epithelial cells, yet they bear little or no fimbriae. This indicates the presence of adhesins on A. actinomycetemcomitans that are not associated with fimbriae. A surfaceassociated protein determined to be an autotransporter has been shown to be involved in A. actinomycetemcomitans adhesion to epithelial cells. The adhesin, termed Aae for adhesion to epithelial cells, is encoded by a gene (aae) that is homologous to autotransporter genes of Haemophilus influenzae and Neisseria species. Thus, the adhesion of A. actinomycetemcomitans to epithelial cells is multifactorial with several adhesins and mechanisms playing a role.

Adhesion to extracellular matrix proteins

The extracellular matrix is a complex network of proteins and polysaccharides that surrounds the cellular components of connective tissues. Collagen is the major protein component of the extracellular matrix. Collagen types I, II, III, V, and XI (fiber-forming types) are predominant in connective tissue, whereas type IV, which differs structurally from the others, is a major component of basement membranes. Fibronectin and laminin, noncollagenous glycosylated proteins, are also found in connective tissue and basement membranes. A. actinomycetemcomitans binds to immobilized collagen types I, II, III, and V, but not to type IV collagen. Binding to collagen types I, II, III, and V does not occur with soluble collagen, suggesting that a specific conformation of the fibrillar collagens is required for binding. A. actinomycetemcomitans outer membrane proteins are essential for the binding to fibrillar collagen. A. actinomycetemcomitans also binds to fibronectin, but not to the plasma protein fibrinogen. Binding, therefore, is highly specific. The binding of A. actinomycetemcomitans to the insoluble form of proteins that are major structural components of the extracellular matrix may aid the organism in its spread and colonization of both oral and extra oral connective tissues.

Invasion of epithelial cells

Early clinical studies showed that A. actinomycetemcomitans can penetrate the gingival epithelium. These in vivo studies revealed that A. actinomycetemcomitans occurs in very specific intracellular locations and exhibits a very specific penetration pattern. In vitro studies have demonstrated that A. actinomycetemcomitans can also invade epithelial cells. Invasion is a dynamic, complex process that involves attachment to the host cell with initiation of A. actinomycetemcomitans uptake in a hostderived membrane-bound vacuole from which it quickly escapes and enters the cytoplasm,

Intracellularly, A. actinomycetemcomitans is not quiescent. A short time after escape from the vacuole into the cytoplasm, it transits through the cell to neighboring cells via bacteria-induced protrusions that appear to be extensions of the host cell membrane. Bacteria can be seen within these protrusions by scanning, transmission, and fluorescent microscopy. Microtubules are strongly implicated in the intra- and intercellular spread of A. actinomycetemcomitans. Internalized A. actinomycetemcomitans interacts with host cell microtubules, In vitro studies show that A. actinomycetemcomitans localizes exclusively with the plus-ends of microtubules of taxol-induced microtubule asters, indicating a specific A. actinomycetemcomitans-microtubule interaction.

On the basis of these in vitro observations, it is proposed that invasion of epithelial cells and the dynamic process of inter- and intracellular spread are the means by which A. actinomycetemcomitans spreads to the gingival connective tissue and initiates the destruction associated with periodontal disease.

Colony phase variation

A. actinomycetemcomitans produces three distinct colonial morphologies on solid medium. Upon primary isolation from the gingiva, it typically forms a rough colony phenotype. These are small (~O.5 to 1 mm in diameter), translucent, rough-surfaced circular colonies with irregular edges that pit the agar. They have a distinctive internal star-shaped or crossed-cigar

morphology from which the genus name of the organism is derived. In liquid medium, the rough colony phenotype cells form aggregates on the vessel walls, thereby leaving the medium clear. Repeated subculture on agar yields two types of colonial variants; one is smooth surfaced and transparent, the other is smooth-surfaced and opaque. The transparent smooth-surfaced variants appear to be an Intermediate between the transparent rough-surfaced and opaque smooth-surfaced types. In contrast to the rough-to-smooth variant transition, which in general occurs rapidly soon after isolation during- in vitro culture, a smooth-to-rough variant transition, which appears to be associated with nutritional factors, occurs only rarely III vitro.

Bacterial colonial variation is indicative of the differential expression of cell surface components. It has been determined that A. actmomycetemcomitans rough colony variants express 43- and 20-kDa outer membrane proteins, rough colony protein A and rough colony protein G. respectively, that are not expressed in smooth colony variants. The genes that encode these proteins have homology to genes known to encode fimbriae-associated proteins. In that regard, A. actinomycetemcomitans rough colony variants are heavily fimbriated, whereas smooth colony variants have few or no fimbriae associated with their surface. Although the role of the phenotypic variation is not known, it has been proposed that it may play some role in the episodic nature of periodontitis.

Interference with host defense mechanisms

Similar to other periodontopathogens, A. actinomycetemcomitans elaborates factors that can modulate and suppress host defense mechanisms. The host's first line of defense against invading bacteria is phagocyte recruitment (chemotaxis) to the region. A number of steps are involved In this process: binding of chemotactic signaling factors, upregulation of adhesion receptors, binding to the endothelium, and movement of phagocytes to the underlying tissues. This is a major host defense mechanism that represents a significant challenge to invading organisms. Thus, the ability to disrupt chemotaxis promotes survival of the organism. A. actinomycetemcomitans can inhibit PMN chemotaxis, and the capsularlike serotype-specific polysaccharide antigen can resist phagocytosis and killing by PMNs. PMNs can also kill bacteria by fusing the phagosome-containing intracellular bacteria with lysosomes from which they acquire potent antibacterial agents. Bacteria able to inhibit the fusion or ward off the anti bactericidal action are protected. A. actinomycetemcomitans can inhibit the production by PMNs of some of these compounds and it is resistant to others. A heat-stable protein in A. actinomycetemcomitans inhibits the production of hydrogen peroxide by

PMNs, and many strains are· intrinsically resistant to high concentrations of hydrogen peroxide. furthermore, A. actinomycetemcomitans is resistant to a number of defensins, cationic peptides that occur in neutrophils.

In addition to phagocytosis and killing, leukocytes and monocytes/macrophages also release biologically active agents, such as cytokines and oxidizing agents, Components of A. actinomycetemcomitans that can induce or enhance the synthesis of cytokines by monocytes include LPS, ISF, serotype-specific polysaccharide antigen, and a 65-kDa antigen. This modulatory activity likely interferes with host immune homeostasis and contributes to the initiation 'and progression of disease.

Bone resorption :

Periodontal disease is associated with loss of alveolar bone, the structure supporting the tooth, A. actinomycetemcomitans can cause bone resorption through at least three different effectors: a surface-associated material (SAM), LPS, and a proteolysis-sensitive factor in, microvesicles, The active component of SAM is the molecular chaperone (heat shock protein), GroEL. This chaperone seems to act directly with osteoclasts, the major bone-resorbing cell population. SA-Yi can also exert an antiproliferative effect on osteoblastlike cells. The mechanism of action of SAM is distinctly different from that of LPS. A, actinomycetemcomitans LPS causes the release of calcium from fetal long bones, Dexamethasone completely inhibits LPS-induced bone resorption activity by a mechanism that likely involves prostaglandins and IL-1. In contrast, GroEL bone resorption activity is not inhibited by IL-l receptor antagonist protein.

Apoptosis

Leukotoxin-mediated killing of promyelocytic HL-60 cells involves the induction of apoptosis through the activation of caspases. Induction of apoptosis by leukotoxin also involves the mitochondrial apoptosis pathway. Removal of acute inflammatory cells by apoptosis may play an essential role in the pathogenesis of diseases mediated by A. actinomycetemcomitans.

Pathogenicity

A number of virulence factors including lipopoly-saccharide (endotoxin), a leucotoxin, collagenase and a protease cleaving IgG have all been isolated from A. actinomycetemcomitans. The leucotoxin, in particular, is thought to play a significant role in subverting the host immune response in the gingival crevice. Together with other coagents A. actinomycetemcomitans is involved in localized aggressive periodontitis and destructive periodontal disease in adults. Also isolated from cases of infective endocarditis, and from brain and subcutaneous abscesses.

MOLECULES AND ORGANELLES

Leukotoxin

Leukotoxin is probably the most studied A. actinomycetemcomitans virulence factor. First described in 1979, this 116-kDa toxic protein was later shown to be produced by about half of the -strains isolated from patients with LAP. A protective role for the antibodies to this toxin is suggested by studies that showed that attachment loss was less in patients with early-onset periodontitis with A. actinomycetemcomitans leukotoxin reactive antibody

than in patients who lacked the antibody. An association between leukotoxin and periodontitis is also suggested by the high levels of leukotoxin in A. actinomycetemcomitans strains isolated from patients with Papillon-Lefevre syndrome, a keratoderma associated with severe periodontitis. Leukotoxin belongs to the RTX family of pore-forming hemolysins/leukotoxins expressed by a variety of pathogenic bacteria. The structural gene for leukotoxin (ltxA) is the second gene of an operon consisting of four genes, C, A, B, and D. Proteins encoded by ltxB and ltxD are involved with transportation of the toxin to the cell surface, whereas the ltxC gene product activates the toxin post-translationally.

Leukotoxin is both species specific (human and primate) and cell specific. It binds only to monocytes, neutrophils, and a subset of lymphocytes, and forms pores in the membranes of these cells. Death can result from the leukotoxin-induced pores interfering with osmotic homeostasis capabilities or through an apoptotic effect. In addition, leukotoxin actually triggers the release of enzymes from PMNs capable of degrading the molecule. However, protease inhibitors within serum can inhibit the proteolytic degradation; thus serum actually enhances leukotoxic activity.

Cytotoxins

Cytolethal distending toxin

(CDT)/immunosuppressive factor (ISF).

CDT is a bacterial heat-labile holotoxin whose biological activities include eukaryotic cell distention and cell cycle arrest, actin rearrangement, and apoptosis; however, the activity elicited depends on the cell type under investigation. ISF produced by A. actinomycetemcomitans is a member of the family of Cdts encoded by the cdtB gene; CdtB protein and ISF are essentially equivalent entities, Three genes, cdtA, cdtB, and cdtC, encode three polypeptides, CdtA, CdtB, and CdtC (27, 29, and 20 kDa, respectively). The functions and the functional relationship(s) of the three proteins have not been clearly defined, However, it is known that CdtB is a type I deoxyribonuclease. A. actinomycetemcomitans CDT has been shown to cause G2 cell cycle arrest in CHO and HeLa cells and in the B-cell hybridoma cell line and lymphocytes. CHO cells were used to show that A. actinomycetemcomitans CdtA anchors the toxin to the cell surface, whereas CdtB and CdtC are responsible for the toxicity. Cdt can stimulate a specific network of cytokines in peripheral blood mononuclear cells; it was shown to cause the production of IL-1β, IL-6, IL-8, and IFN-γ, but not of IL-12, TNF-α, or granulocytemacrophage colony-stimulating factor. The inhibition of proliferation and cytokine induction both likely contribute to the pathogenesis of A. actinomycetemcomitans. Leukotoxin and Cdt can also induce apoptosis in human immune cells, which would disrupt immune surveillance.

LPS

The high-molecular-mass 0 polysaccharide component of A. actinomycetemcomitans LPS harbors the A. actinomycetemcomitans immunodominant antigen. It is known that A. actinomycetemcomitans LPS causes bone resorption, platelet aggregation, and skin necrosis. A. actinomycetemcomitans LPS has also been shown to bind strongly to hemoglobin, a source of iron for its growth. In addition, A. actinomycetemcomitans LPS can activate macrophages. At low concentrations it stimulates macrophages to produce IL-1α, IL-1β, and TNF, cytokines that are involved in. tissue inflammation and bone resorption. At higher doses LPS stimulates the production of both proinflammatory and anti-inflammatory cytokines in whole blood. It has been proposed that the ratio of the production of the two types of cytokines may affect the outcome of periodontal diseases.

Fc-binding proteins

The binding of antibody to specific receptors on PMNs is mediated by the Fc region of the antibody molecule. Bacterial Fc receptors (immunoglobulin [Ig]-binding proteins) are proteins associated with, or released from, the cell surface that can bind to the Fc region of Igs. These proteins can thus compete with PMNs for binding to the Fc region and in so doing inhibit phagocytosis. Fc-binding activity is associated with a member of the OmpA family of gram-negative heat-modifiable membrane proteins and with surface capsular material. Since Fc receptors are also believed to play a role in complement activation, the A. actinomycetemcomitans Fcbinding protein(s) would inhibit both of these important host defense functions.

Membranous vesicles

Vesicles (blebs) are a prominent feature of the surface of A. actinomycetemcomitans. These structures either remain attached to the cell surface or bud off from it. Large numbers of vesicles are released into the external environment. Vesicles associated with A. actinomycetemcomitans grown on agar are thick fibrils with knob-like ends. Highly leukotoxic A. actinomycetemcomitans strains have an abundance of vesicles, whereas low- or non leukotoxic strains have few or no vesicles. In addition to leukotoxin, A. actinomycetemcomitans vesicles also possess LPS with endotoxin activity, bone resorption activity, and actinobacillin, a bacteriocin. A. actinomycetemcomitans vesicles must also contain adhesins as the addition of vesicles to weakly adherent or nonadherent strains significantly increases the ability of those strains to attach to epithelial cells. The observation that A. actinomycetemcomitans vesicles exhibit adhesiveness prompted the hypothesis that vesicles can also function as delivery vehicles for A. actinomycetemcomitans toxic materials.

Extracellular amorphous material

The surfaces of certain A. actinomycetemcomitans are associated with an extracellular amorphous material (ExAmYlat), which actually embeds adjacent cells in a matrix. It has been determined that ExAmMat is proteinaceous, most likely a glycoprotein, with both bone resorbing activity and adhesive properties. Moreover, when A. actinomycetemcomitans strains that normally adhere weakly to epithelial cells are suspended in ExAmMat, they adhere tightly, a phenomenon termed

conveyed adhesion.

Fimbriae

A. actinomycetemcomitans fimbriae are peritrichous, may be ~2μm in length and 5 nm in diameter, and frequently occur in bundles. Freshly isolated strains are fimbriated, but in vitro subculture results in organisms that, in general, lack or have few fimbriae. A. actinomycetemcomitans adhesiveness to epithelial cells and to both hydroxyapatite and saliva-coated hydroxyapatite is associated with colonial variation and fimbriation. Fimbriae contain a 54-kDa fimbrial subunit (fimbrial associated protein) that is involved in adherence. An additional 6.5-kDa protein, Flp, can also be present, and this exhibits some amino acid sequence similarity to type IV pilin. Clearly there is a correlation between A. actinomycetemcomitans fimbriation and adhesion; however, nonfimbriated A. actinomycetemcomitans also exhibits adhesive properties, indicating that nonfimbrial components also function in adhesion.

Role in periodontics

Localized aggressive periodontitis

The AAP classified periodontitis into aggressive periodontitis and chronic periodontitis.

These forms of periodontitis were further sub classified into generalized and localized based on the percentage of sites involved.

Formerly known as localized juvenile periodontitis (LJP), localized aggressive periodontitis (LAP) involves a peculiar form of periodontitis where destruction is restricted to the first molars and incisors, and is characterized by a symmetrical distribution, contrary to the generalized form, where almost all teeth are involved.

Prevalence of LAP

Several studies have been performed to investigate the prevalence of LAP. Neely et al [1983-84] found a prevalence of 0.46%while Loe found a prevalence of 0.53% .Black were more likely to develop LJP then whites, Black males were 2.9times as likely to have LJP as compared to black females. Tinoco et al conducted a study on a Brazilian population and found an overall prevalence of 0.3%.

Clinical characteristics

After initial colonization of the 1st permanent teeth erupt (1st molars and incisors). A.a evades the host defenses by different mechanism, including production of PMNs chemotaxis inhibiting factors, endotoxin, collagenases, leukotoxin and other factors. It allow to bacteria to colonize the pocket and initiate the destruction of periodontal tissues. Bacteria antagonistic to A.a may colonize the periodontal tissues and inhibit A.a from further colonization. This would localize A.a infection and tissue destruction. A.a may lose its leukotoxin producing ability for unknown reason. Progression of disease may become arrested. Defect in cementum formation may be responsible for localization of the lesions.

Conclusion

A. actinomycetemcomitans is a bacterium with an array of diverse potential virulence characteristics, including multiple immune evasion mechanisms and novel mechanisms for binding to host matrices and invading host cells, any one of which may play a crucial role in the local tissue pathology of LAP. Our understanding of this organism still lags behind that of enteric pathogens, largely because methods for genetic manipulation have only just become available and the genome sequence, while almost complete, still awaits annotation.

With the availability of such methodology and genome information we should begin to see rapid advances in understanding how A. actinomycetemcomitans produces such profound, but local, pathology and shed light on its ability to induce systemic pathology such as the recent report of glomerulonephritis caused by this bacterium.

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