ROLE OF ACTINOBACILLUS ACTINOMYCETEMCOMITANS IN PERIODONTAL DISEASES.

1Asha Latha Tella
2Sreenivas Nagarakanti
3Jana Kumar Amulum

1 2 3 Department of Periodontics, Narayana Dental College and Hospital, Nellore, Andhra Pradesh, India

ABSTRACT: Actinobacillus actinomycetemcomitans (AA comitans) is an important pathogenic microorganism of various periodontal diseases. The pathogenic mechanisms of AA comitans and its role in Periodontal diseases is discussed here.

KEYWORDS: Actinobacillus actinomycetemcomitans, Periodontal diseases, pathogenesis

INTRODUCTION

Periodontal disease comprises a group of inflammatory conditions of supporting tissues of the teeth that are caused by bacteria. Our understanding of the etiology of periodontal diseases has undergone major advances in recent decades.

Periodontal diagnosis and treatment often requires a thorough understanding of complex pathobiology of periodontal disease.

In 1976 Walter Loesche, a researcher at the University of Michigan proposed "Non Specific Plaque Hypothesis. According to this, periodontal disease results from the "elaboration of noxious products by the entire plaque flora."

Later Loesche proposed the "Specific Plaque Hypothesis" which states that only certain plaque is pathogenic and its pathogenicity depends on the presence of or increase in specific organisms. Acceptance of the specific plaque hypothesis was spurred by the recognition of "Actinobacillus actinomycetemcomitans " as a pathogen in Localized Aggressive Periodontitis.

Actinobacillus actinomycetemcomitans is an exogenous bacteria that causes true infections and are transmissible among exposed individuals. It is a major putative periodontopathic bacteria. It has been closely associated with periodontitis in young individuals and with cases of refractory periodontitis. The prevalence of Actinobacillus actinomycetemcomitans is nearly 90% in Localized Aggressive Periodontitis and 30-50% in severe adult periodontitis and is also frequently associated with rapidly progressive periodontitis. It can also seed to and produce severe infections in extroral sites.

The morphological and cultural characteristics of Actinobacillus actinomycetemcomitans was first described by Klinger. This gram-negative, facultatively anaerobic rod was first isolated from a cervicofacial actinomycotic lesion in 1912 and initially designated as Bacterium actinomycetemcomitans. In 1921, the organism was referred to as Bacterium comitans by Lieske and finally was designated as Actinobacillus actinomycetemcomitans in 1929.

The term Actinobacillus connotes the resemblance of a ray fungus and also refers to the internal star-shaped morphology. The name actinomycetemcomitans arises from its association with an actinomycete and frequent isolation from actinomycotic lesions.

Actinobacillus actinomycetemcomitans is a member of the genus Actinobacillus that belongs to the family "Pasteurellaceae." Slots studied 135 biochemical characters in 6 reference strains and 130 strains of Actinobacillus actinomycetemcomitans in his study biochemical reaction pattern of Actinobacillus actinomycetemcomitans was remarkably uniform.

King and Tatum provided the first detailed biochemical, serological description of Actinobacillus actinomycetemcomitans. Using tube agglutination studies Taichman classified Actinobacillus actinomycetemcomitans into five serogroups and designated as a, b, c, d and e. Serotypes a, b and c are most prevalent in oral cavity. Healthy subjects frequently carry serotype c strains.
Reclassification of Actinobacillus actinomycetemcomitans - Haemophilus aphrophilus, Haemophilus - paraphrophilus and Haemophilus segnis as Aggregatibacter actinomycetemcomitans gen. nov., comb. nov., Aggregatibacteraphrophilus comb. nov. and Aggregatibacter segnis comb. nov., and emended description of Aggregatibacter arophrophilus.9

Morphological Characteristics

Morphological Characteristics of Actinobacillus actinomycetemcomitans were first described by klinger. Actinobacillus actinomycetemcomitans is a Gram-negative coccobacillus approximately 0.4 x 0.1 micrometers in size. Microscopically, culture appears predominantly bacillary with a few coccoid forms. It is capnophilic, requiring an atmosphere containing 5-10% CO2 for good growth. It is microaerophilic and a facultative anaerobe and can grow under anaerobic conditions. It is nonsporulating, non-motile, non-hemolytic, oxidase and catalase positive.6

A significant feature of Actinobacillus actinomycetemcomitans is its surface ultrastructure, which includes fimbriae, vesicles and extracellular amorphous material.

Implication of Actinobacillus actinomycetemcomitans in Periodontal Disease

Actinobacillus actinomycetemcomitans is an important pathogen in severe and recurrent forms of periodontitis. Prevalence of Actinobacillus actinomycetemcomitans is nearly 90% in Localized Juvenile Periodontitis and 30-50% in severe Adult periodontitis. It is frequently associated with rapid and progressive periodontitis. Serotype B strains are often predominant in periodontal lesions of Localized Juvenile Periodontitis patients.8

Genetic system of Actinobacillus actinomycetemcomitans

Isolation of a plasmid from Actinobacillus actinomycetemcomitans is key in the construction of inter-generic plasmids for the development of genetic transfer systems. The observation of plasmids in Actinobacillus actinomycetemcomitans was first documented from 10 clinical isolates from periodontal lesions of patients with rapidly destructive periodontitis.11 Identical four plasmids of various molecular sizes (4-20kDa) were isolated. Of thirty-nine isolates examined, only two of the strains harbored detectable plasmids. One strain contained two plasmids, 1.9-kb and greater than 30-kb, and the other strain harbored a single 24-kb plasmid. The 1.9-kb plasmids are designated as pVT736-1.

Development of cloning vectors

The absence of a selective phenotype of the naturally occurring plasmid pVT736-1 made it unsuitable for use in a transformation system with out further manipulation. A recombinant plasmid containing two antibiotic resistant markers were developed subsequently by ligation with the Escherichia coli plasmids pUC19 or pGEM7Zf. The plasmid obtained was pDL282. Two unique deletion derivatives of this recombinant plasmid were obtained and are designated as pPK1 (3.6kb) and pPK2 (2.5kb). Both were stably maintained in Actinobacillus actinomycetemcomitans when grown in the presence of spectinomycin and were restricted when placed in ampicillin, which indicates that the ampicillin gene denoted as bla was deleted from the parent plasmid.

The instability of pDL282 precluded the use of this plasmid as a cloning vector. The smaller sized pPK1 functioned effectively as a cloning plasmid.

Gene transfer systems Transformation

Transfer of genetic material into Actinobacillus actinomycetemcomitans was first achieved by electroporation.12 The transformation efficiency varied over four orders of magnitude with the strain of Actinobacillus actinomycetemcomitans and the chimeric plasmid used. Interestingly, a spontaneous smooth-colony derivative was transformed at a lower efficiency than their rough-colony parents with two of the chimeric plasmids. Most of the plasmids tested contained bla (ampicillin gene) as the selectable marker. Several strains of Actinobacillus actinomycetemcomitans tested were found to be ampicillin resistant when pUC19 was used for transformation.

Conjugation

Transfer of genetic material into Actinobacillus actinomycetemcomitans has also been accomplished by conjugation. Conjugation is a form of bacterial 'mating' in
which genetic material is transferred from one bacteria to another. It is mediated by genetic information encoded by plasmid or chromosomal determinants. Goncharoff et al demonstrated the first example of conjugal transfer of DNA from Escherichia coli to Actinobacillus actinomycetemcomitans.

Advances in the molecular genetics of Actinobacillus actinomycetemcomitans

The development of intergeneric plasmids and efficient transformation systems provides the tools that are necessary to dissect the molecular mechanisms of the regulation and expression of virulence factors synthesized by this pathogen. Actinobacillus actinomycetemcomitans produces a leukotoxin that specifically lysed human and primate polymorphonuclear leukocytes. The leukotoxin is a 116-kDa protein and is a member of the RTX toxin family of proteins. The lkt operon is composed of the leukotoxin structural gene (lktA) and three other genes, lktC, lktB, lktD, which code for proteins, that are involved in the activation and localization of the leukotoxin protein.

Mutagenesis of lktA by insertional duplication generated a strain of Actinobacillus actinomycetemcomitans, which expressed leukotoxin activity at <0.1% of the activity of the parent strain. Inactivation of lktBD also reduced the leukotoxin activity in the mutant strains, but had no effect on the level of leukotoxin RNA. This data indicate that genes are involved in leukotoxin synthesis post-transcriptionally.

The strains producing low levels of toxin use lkt operon as the promoter for transcription. The promoter sequences and lacZ reporter gene were cloned into a broad-host-range plasmid IncQ, transformed into Actinobacillus actinomycetemcomitans by electroporation and the β-galactosidase levels determined. The results indicated that the 530bp segments found in low-leukotoxin strain is a sequence involved in down regulation of transcription of leukotoxin gene.

Southern blot analysis of the transformants suggested that the transposon be inserted into a variety of different sites on the chromosome.

Virulence Factors

Factors that promote colonization and persistence in the oral cavity
- Adhesins
- Invasins
- Bacteriocins
- Antibiotic resistance
- Factors that interfere with the host’s defenses
  - Leukotoxin
  - Chemotactic inhibitors
  - Immunosuppressive proteins

Factors that destroy host tissues
- Cytoxins
- Collagenase
- Bone resorption agents
- Stimulators of inflammatory mediators
- Factors that inhibit host repair of tissues
  - Inhibitors of fibroblast proliferation
  - Inhibitors of bone formation

CONCLUSION

Further progress in the management of periodontal disease depends on better understanding of the periodontopathic microorganisms, their epidemiology and how they cause disease.

It is of considerable interest to know that Actinobacillus actinomycetemcomitans possess so many virulence factors, but unfortunately only a few have been extensively studied. If we hope to understand and eradicate this pathogen, it is critical then in-depth investigations into biochemistry, genetic expression, regulation and mechanisms of action of these factors be initiated.

References

4. Lieske R – Morphology and Biologie der Strahlenpilze; Leipzig; Borntraeger, 1921.
8. Shan-ling H etal - inhibitory effects of arecanut extracts on phagocytosis of Actinobacillus


11. Olsvik B, Preus HR - Plasmids in Actinobacillus actinomycetemcomitans strains isolated from periodontal lesions of patients with rapidly destructive periodontitis; Oral Microbiol Immunol 1989; 4; 219 – 221.


Corresponding Author

Dr. Asha Latha Tella
Reader,
Department of Periodontics
Narayana Dental college,
Nellore
Phone No: 9989481600
Email: drasha_23@yahoo.com