Research & Reviews: A Journal of Dentistry
Volume 1, Issue 1, November, 2010, Pages 1-10.

An Update on the Virulence Factors of Actinobacillus actinomycetemcomitans - A Systematic Review

Dr. Shikha Kler[*] *and Dr. Rajvir Malik*[¶]

*Department of Oral and Maxillofacial Pathology, ¶ Department of Periodontology and Oral Implantology, DAV(C) Dental College, Yamuna Nagar, Haryana- 135001, India

ABSTRACT

Over the last 20 years, research on *Actinobacillus actinomycetemcomitans* (*Aa*) a periodontopathogen, has provided a tremendous amount of information as a major anaerobic, opportunistic pathogen. Elucidation of the virulence in *Aa* is especially difficult because of this bacterium's pathogenicity being multifaceted, including both microbial and host factors and the inability to clearly define the disease state of many forms of destructive periodontal disease. The objectives of this study were to review the virulence factors of *Aa* considering the unique features of *Aa* when compared with other gram-negative bacteria and explicate the general aspects of the virulence factors of *Aa* and their mechanism in particular. A comprehensive search strategy of electronic databases (MEDLINE, EMBASE, The Cochrane Central Register of Controlled Trials, the U.K. National Research Register and Conference Proceedings Citation Index) using key words was performed. In addition, hand searches were made for several dental, microbiological and immunity journals not cataloged in the databases, and the reference lists from published articles were checked. It was concluded that virulence factors of *Aa* allows it to make contact with host tissues and cells, which are potentially important in the incidence of periodontal diseases.

Keywords: Adhesions, Collagenase, Genes, Leucotoxin, Surface-associated material

[*]Author for correspondence: shikharnav@yahoo.com

INTRODUCTION

Periodontal disease is a significant global public health concern and is probably the most common chronic infectious disease of humans.(1,2) The pathogenesis of periodontitis is characterized by microbial challenge and the host's response to it.(3) From the approximately 500 bacterial species colonizing periodontal pockets and a further 300 in the rest of the oral cavity, approximately one half still awaits growth in culture. From the other half some bacteria are always closely associated with periodontal destruction.(4) Despite the complex parasite-host interactions in human periodontal

disease, a considerable body of evidence points to *Aa* as an important periodontal pathogen.(5) The designation of *Aa* as periodontal pathogens presupposes that destructive periodontal disease is more prevalent in periodontal sites exposed to the microbes than in non exposed periodontal sites. It also assumes that future tissue destruction takes place more frequently in periodontal sites exposed to the microbes than in those not exposed.(6)

Actinobacillus actinomycetemcomitans (now termed as *Aggregatibacter actinomycetemcomitans*) a facultative gram negative,(4-6) capnophilic, fermentative, coccobacillus **(**1**,**3-6) appears to play an important(7) role in aggressive periodontitis(8) [previously referred to as early onset(9)/juvenile(10)/ rapidly progressive periodontitis (10)] and upto some extent in chronic(8) [adult periodontitis (9)] periodontitis. Although the pathogenic mechanism by which this bacterial species acts to cause periodontal disease is not known, this organism is able to produce a variety of virulence factors capable of facilitating the colonization, invasion and destruction of the periodontal tissues and interfere with tissue repair. (11)

Virulence factors

Virulence is defined as the ability of a microbe to cause infection. Generically, the virulence attributes of microbial pathogens include the ability to enter a host, find a unique ecological niche, Circumvent or subvert the host's normal defenses, replicate in the new environment, express specialized pathogenic traits.(1)

Virulence is recognized depending on synchronized expression of several genes whose products mediate attachment as well as provide ability to escape host defenses, production of tissue destroying toxins and enzymes(1) etc. The various virulence factors of *Aa* are leucotoxin which can destroy PMNs and monocytes,(11) cytolethal distending toxins,(1) lipopolysaccharides [LPS](12), Surface-associated material [SAM],(13) chemotactic inhibition factors,(14) proteases that degrade immunoglobulins,(12) collagenase which may degrade connective tissue collagen,(15) extracellular outer membrane vesicles,(12) factors affecting the immune response,(17) factors damaging host cells including epithelial cells and fibroblasts,(18) virulence traits relevant to tissue penetration: adhesins and fimbriae.(1)

a) Leukotoxins (LtxA)

The *Aa* leukotoxin (19) is a member of a family of pore-forming toxins, characterized by a series of glycine- rich repeats in the C-terminal portion of the protein that are involved in cation binding and appear to be essential to toxin activity. This family

has been referred to as *RTX (repeat in toxin).* The repeat-in-toxin exoprotein family is found among numerous genera of gram-negative bacteria and may play a significant role in periodontal disease pathogenesis.(19,20)

The leukotoxin appears to be mainly present in the outer cell membrane,in the extracellular outer membrane vesicles:

Aa strains vary in their ability to produce the leukotoxin and the strains can be classified (19) into leukotoxin producing – strains and nonleukotoxin-producing strains.

The mechanism of leukotoxicity(21) includes:

- Membranolytic activity producing pores in the target cell.
- Phospholipids act as the receptor for the toxin whose activity result in a rapid influx of Ca^{2+} into the cell.
- Necrosis and apoptosis.

Exposure of neutrophils and monocytes/ macrophages, to strains that produce large amount of LtxA, result from the ability of LtxA to form pores in the membrane of the target cells, leading to osmolysis caused by water influx into the cells.

In contrast, prolonged exposure of lymphocytes and NK cells to LtxA, results in apoptosis. Lower concentration of LtxA result in apoptosis whereas higher concentration results in necrosis.(22)

Molecular Characterization of the Leukotoxin Genes

The gene encoding the *Aa* leukotoxin, ltxA, is part of operon of four genes in the sequence ltxA, ltxB, ltxC, ltxD. Zambon et al. found a high leukotoxin activity in *serotype b* and low or none in other serotypes.(23)

This toxin is a 116-kDa protein produced by 56% of strains isolated from localized juvenile periodontitis patients.(24) Early-onset periodontitis patients with antibody reactive with *Aa* leukotoxin exhibit decreased attachment loss compared with patients who lack the antibody suggesting a protective role for the antigen.(1, 24)

The cytolethal distending toxin (cdt) genes

Cytolethal distending toxin is encoded by a locus of three genes, cdtABC.(25,26) The toxin itself is encoded by cdtB, while cdtA and cdtC appear to encode proteins that mediate interaction between the cytolethal distending toxin complex and the host cell surface. Several reports indicate that the Cdt complex of *Aa* is composed of all three proteins CdtA, -B and -C, in equal proportions(27)While CdtB exhibits toxicity when artificially injected into cells all three proteins are required for maximal toxin activity.(3)

Mechanism of action

The active subunit, CdtB, exhibits DNase I activity. While the role of CdtA and CdtC is less clear, both proteins possess putative mucin-like carbohydrate-binding domains that predict interaction with the host cell surface.

Indeed, CdtA and CdtC have been shown to bind directly to Chinese hamster ovary cells. Thus, the current model for cdt action predicts that CdtA and CdtC interact with the host membrane and facilitate CdtB entry into the cell. After CdtB enters the cell, it is transported into the nucleus by an active process that requires amino acid residues in its N terminus.(3)

In the nucleus, *CdtB* causes

DNA damage through its DNase activity

↓

Induction of apoptosis.

Induction of apoptosis in lymphocytes occurs through capase activation.

In human gingival fibroblasts, *Aa* Cdt is able to stimulate the production of receptor activator of nuclear factor-KB ligand, and this activation is independent of interleukin-1, interleukin-6, tumor necrosis factor-α, or prostaglandin E_2 expression.

Belibasakis et al.(21) found that *Aa* Cdt is largely responsible for the inhibition of proliferation of human periodontal ligament cells and gingival fibroblasts. This effect could seriously affect the physiology of the periodontium and exacerbate disease. Additionally, induction of receptor activator of nuclear factor-KB ligand by Cdt may be involved in pathological bone resorption, characteristic of localized aggressive periodontitis.

b) Lipopolysaccharide (LPS)

It is a major integral component of the outer membrane of gram negative bacteria.(5) The LPS of *Aa* has a broad spectrum of immunological, endotoxic activities.

These activities include

- Stimulation of *in vitro* bone resorption.
- The production of IL-1, and prostaglandin (PGE_2) from macrophages.
- Polyclonal activation of B-lymphocytes.(25)

The bone resorptive activities of this LPS (28,29) are the result of stimulation of PGE_2 ,IL-I release from osteoblasts and other cells.

Aa is known to activate the complement cascade by the alternative pathway which in turn generates prostaglandins and this is the probable mechanism of bone resorption in case of periodontitis.(30)

c) Surface–associated material (SAM)

It has been shown that proteins associated with the outer surfaces of some but not all putative periodontal pathogens are potent inducers of bone resorption and tissue pathology in vitro.(31)

The SAM(29) of *Aa* is composed

- Bacterial capsule and
- Other molecules loosely bound to the outer surface of the external membrane.
- Several proteins and peptides.

These proteins are active in very low concentrations and it is presumed that their actions are important in the pathogenesis of periodontitis by stimulating alveolar bone resorption. These inhibit:

- Periodontal ligament regeneration and repair.
- Promote B-lymphocytes and plasma cell proliferation.
- Stimulate T suppressor cells.
- Suppress immunoglobulin production.
- Resistance to complement - mediated killing.
- Escape the anti-bacterial actions of the immune system by surviving within epithelial cells and other periodontal mammalian cells.

There is some evidence that SAM could directly stimulate the proliferation and differentiation of osteoclasts. Indirectly they do this by stimulating osteoblasts to produce signals, other than the cytokines or prostaglandins.(32)

SAMs are much more potent bone-resorbing agents than lipopolysaccharides. In this regard SAM is 1000 times more potent than the corresponding LPS.(29,33)

d) Chemotactic inhibition factors

A.actinomycetemcomitans produces factors which inhibit the chemotaxis of PMNs.(34,35) These are known as chemotactic inhibition factors. These factors could reduce the number of PMNs in the local lesion available to phagocytose and kill these bacteria.

e) Extracellular outer membrane vesicles

Aa produces numerous extracellular outer membrane vesicles which are shed from the surface of the bacteria. These vesicles contain

- leucotoxin
- LPS

Their small size permits them to cross epithelial barriers such as the pocket epithelium.(36)

f) Factors affecting the immune response

Aa produces a potent polyclonal B- lymphocyte-activating factor(37) which may contribute to the pathogenesis of the condition by:

- Inducing B-Lymphocytes to produce antibodies with determinants unrelated to the bacterial antigens.

This may in part be due to the LPS and SAM proteins present in outer membrane vesicles.(14)

g) Factors damaging host cells

Aa produces an

a) **Epitheliotoxin**
b) **Fibroblast-inhibiting factor**

a) Epitheliotoxin can damage :

- epithelial cells
- facilitate bacterial penetration of the junctional and pocket epithelium.
- may aid in the invasion of *Aa* into epithelial cells.

This may be a mechanism by which it might evade the host defenses and may explain the episodic nature of this disease.

In this regard these bacteria have been found in the connective tissue in contact with collagen and fibronectin and these proteins may be potential binding sites of *Aa*.(29)

b) Fibroblast-inhibiting factor may impair tissue repair.

Specific attachment of *Aa* to host tissues is critical for infection and these bacteria adhere to and invade into epithelial cells.(29)

h) Proteases that degrade immunoglobulins

A.actinomycetemcomitans produces proteolytic enzymes which degrade immunoglobulins**.** This could reduce the local effectiveness of antibodies produced against these bacteria.(29)

(i) Collagenase *Aa* produces a collagenolytic proteinase which can attack collagen. This could contribute to degradation of collagen and connective tissue breakdown in the periodontal tissues. In this regard an arginine- and lysine-specific protease of approximately 50 kDa in molecular weight has been purified from the culture supernatant of *Aa* and this enzyme showed collagen degrading activity.(18,38)

The purified protease (38) has also been shown to reduce the cell growth rate, DNA synthesis rate and fibronectin level of human gingival epithelial cells in a dose – dependent way in vitro. Thus these proteases may inhibit the proliferation.

(ii) Adhesins are proteinaceous in nature and mediate the attachment of bacteria to specific receptors on epithelial cells. These are

- Associated with the outer membrane of the bacterium or
- Are released into the medium in the form of vesicles.

The identities of these molecules are unknown and the relationship between the bound and secreted adhesion molecules is yet to be determined.

Fimbriae gives the bacteria a rough morphology, the morphological type associated with fresh isolates adhesion of *Aa* to epithelial cells involves multiple determinants. *Aa* exhibits more fimbriae when grown anaerobically than when cultured in CO_2.

Like many other gram-negative bacteria, *Aa* may exhibit fimbriae, small filamentous cell surface appendages associated with bacterial colonization of host tissues.(16) *Aa* fimbriae (16,39) occur in peritrichous arrays,(40) may be more than 2 pm in length and 5 nm in diameter and often occur in bundles.(41,42)

Recently, it was reported that the most abundant protein in a fimbria preparation of A. *actinomycetemcomitans* was a protein with an approximate apparent molecular mass of 6.5k Da; only a small amount of the 54-kDa protein was present. The low-molecular-weight protein, termed Flp, exhibits some amino acid sequence similarity to type-IV pili.(43)

These have all been suggested for their potential involvement in *Aa*-induced pathogenesis of periodontal disease.(26)

(iii) Vesicles

A prominent feature of the surface of *Aa* is vesicles (blebs). These structures, which are lipopolysaccharide in nature, originate from and are continuous with the outer membrane. Vesicles are also released into the external environment in large numbers.(40) The surface of highly leukotoxic *A. actinomycetemcomitans* strains has an abundance of extracellular membranous vesicles, in contrast to minimally or non-leukotoxic strains, which have few or no vesicles.(44) Furthermore, vesicles *per se* exhibit leukotoxic activity(41). Other biologically active components of *A. actinomycetemcomitans* vesicles are endotoxin,(46) bone resorption activity(46) and a bacteriocin, termed actinobacillin.(12)

i) Bone resorption

A characteristic feature of periodontal disease is the loss of bone supporting the teeth. *Aa* has been shown to stimulate bone resorption by several different mechanisms: lipopolysaccharide(45),

proteolysis-sensitive factor in microvesicles(47) and surface-associated material.(48) Surface-associated material has recently been identified as the molecular chaperone, GroEL. The chaperone appears to act in a direct way with the major bone-resorbing cell population, the osteoclast(49). The mechanism of action is believed to be distinctly different from that observed with lipopolysaccharide. *Aa* lipopolysaccharide, also a very effective bone resorption mediator, has been shown to cause the release of calcium from fetal long bones in the (44) Ca^{2+} fetal bone resorption assay(45). The bone resorption activity is completely inhibited by dexamethasone and probably involves prostaglandin and interleukin-1.(50) This is in direct contrast to GroEL, whose bone absorption activity is not inhibited by interleukin- 1 receptor antagonist protein.(49)

CONCLUSION

Identifying the virulence factors of *Aa* involved in the pathogenesis of periodontal disease is the most difficult task. Difficulties arise from

- Bacterial pathogenicity being multifaceted, including both microbial and host factors.
- Inability to clearly define the disease state of many forms of destructive periodontal dis-ease.

The tissue-destructive potential of *Aa* may include the production of toxins and enzymes or the induction of immunopathological reactions.

Aa produces a variety of factors which could increase its virulence and potentially damage the tissues of the host.

Surface components of *Aa* are potent stimulators of bone resorption and can induce the release of a range of cytokines which can initiate tissue destruction. A number of surface components can also inhibit the proliferation of fibroblasts and their production of components of the extracellular matrix. Little is known, however, regarding the way in which these factors operate in vivo to produce the pathological features of the disease.(36)

In this part of the world there is a saying that you don't get anything for free but *they (bacteria) live with us and on us, they were here before us and will be here after us.* As a single cell creature they have figured out how to live and survive in complex competitive communities. They have learned how to mimic many things we humans express and in some cases, we cannot distinguish between them and us.(26)

REFERENCES

1. Fives-Taylor P. M. et al. *Periodontology 2000* 1999. 20. 136-67p.
2. Brown L. J. et al. *Journal of Dental Research* 1996. 75(spec issue). 672-83p.
3. Feng Z. and Weinberg A. *Periodontology 2000* 2006. 40. 50-76p.
4. Moore W. E. C. and Moore L. V. H. *Periodontology 2000* 1994. 5. 66-77p.
5. Slots J. and Ting. M. *Periodontology 2000.*1999. 20. 82-121p.
6. Tan S. K. et al. *Journal of Periodontal Research* 2002. 37(4). 268-72p.
7. Kornman K. S. and Loe. H. *Periodontology 2000* 1993. 2. 83- 97p.
8. Armitage G. C. *Annals of Periodontology* 1999. 4. 1-6p.
9. Attstrom R. and Vander Velden. U. *Summary of session 1. In Lang N, Karring T(eds): Proceedings of the 1st European workshop in periodontology* 1993. Berlin, Quintessence.
10. Caton J. *American Academy of Periodontology* 1989.1-32p.
11. Tsai C. C. et al. *Infection and Immunity* 1984.43(2).700-5p.
12. Kiley P. and Holt. S. C. .*Infection and Immunity* 1980. 30(3). 862-73p.
13. Kamin S. et al. *Journal of Medical Microbiology* 1986. 22(3). 245-9p.
14. Van-Dyke T. E. et al. *Journal of Periodontology online* 1982. 53(8). 502-8p.
15. Robertson P. B. et al. *Journal of Periodontal Research* 1982. 17(3). 275-83.
16. Holt S C. et al. *Infection and Immunity* 1980. 30(2). 588-600.
17. Shenker B. J. et al. *Journal of Immumology* 1982. 128(1). 148-54.
18. Slots J. et al. *Infection and Immunity* 1980. 29(3). 1013-20.
19. Haraszthy V. I. et al. *Journal of Periodontology online* 2000 71(6). 912-22.
20. Gmur R. and Baehni P. C. *Oral Microbiology and Immunology* 1997. 12(1). 1–10.
21. Slots J. *Periodontology 2000* 1999. 20. 7-13.
22. Korostoff J. et al. *Infection and Immunity* 1998. 66(9). 4474-83p.
23. Zambon J. J. et al. *Infection and Immunity* 1983. 40(1). 205-12p.
24. Califano J. V. et al. *Oral Microbiology and Immunology* 1997. 12(1). 20-6p.
25. Sugai M. et al. *Infection and Immunity* 1998. 66(10). 5008-19p.
26. Fine D. H. et al. *Periodontology 2000* 2006. 42(1). 114-57p.
27. Saiki K. et al. *Journal of Biochemistry* 2004. 136(3). 335-42p.

28. Belibasakis G. N. et al. *Infection and Immunity* 2005. 73(1). 342-51p.
29. Eley B. M. et al. *Early– onset periodontitis: Periodontics*. 2004. Wright Publishing Company. TX 332-44p.
30. Rogers J. E. et al. *Journal of Periodontology online* 2007. 78(3). 550-8p.
31. Meghji S. et al. *Journal of Periodontology online* 1992. 63(9). 736-42p.
32. Kamin S. et al. *Journal of Medical Microbiology* 1986. 22(3). 245-9p.
33. Zubery Y. et al. *Infection and Immunity* 1998. 66(9). 4158-62p.
34. Astemborski J. A. et al. *Journal of Periodontology online* 1989. 60(10). 557-63p.
35. Van Dyke T. E. et al. *Journal of Periodontology online* 1982. 53(8). 502-8p.
36. Mayrand D. and Grenier D. *Canadian Journal of Microbiology* 1989. 35(6). 607-13p.
37. Bick P. H. et al. *Infection and Immunity* 1981. 34(1). 43-9p.
38. Wang P. L. et al. *European Journal of Oral Sciences* 1999. 107(2). 147-53p.
39. Beachy E. H. ed. *General concepts and principles of bacterial adherence in animals and man.* Bacterial adherence1980. Chapman & Hall. London.
40. Scannapieco F. A. et al. *Infection and Immunity* 1987. 55(9). 2320-3p.
41. Preus H. R. et al. *Oral Microbiology and Immunology* 1988. 3(2). 93-4p.
42. Rosan B. et al. *Oral Microbiology and Immunology* 1988. 3(2). 58-63p.
43. Finlay B. B. and Falkow S. *Microbiology and Molecular Biology Reviews.* 1997. 61(2). 136-69p.
44. Lai C. H. et al. *Journal of Periodontal Research* 1981. 16(4). 379-89p.
45. Hammond B. F. et al. *Journal of Dental Research* 1981. 60. 333p.
46. Nowotny A. et al. *Infection and Immunity* 1982. 37(1). 151-4p.
47. Hammond B. F. et al. *Infection and Immunity* 1987. 55(3). 686-91p.
48. Meghji S. et al. *Immunology and Medical Microbiology.* 1995. 10. 101-8p.
49. Meghji S. et al. *Journal of Medical Microbiology.* 1994. 41. 197-203p.
50. Ishihara Y. et al. *Journal of Periodontal Research* 1991. 26. 155-60p.

