Review Article

International Journal of Dental and Health Sciences
Volume 01.Issue 02

AN INSIGHT IN AGGREGATIBACTER ACTINOMYCETEMCOMITAN LEUKOTOXIN: A REVIEW

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ABSTRACT:

Aggregatibacter actinomycetemcomitans is a gram-negative bacterium that is present in the oral cavity of a large proportion. Human leukocytes are selectively killed through leukotoxin produced by the bacteria. Leukotoxin belong to Repeat in Toxin (RTX) family it is a large pore-forming protein. It effects the host cell in various ways. It interacts with the target host cell receptor, LFA-1, which is expressed on leukocytes. A. actinomycetemcomitans is a major etiologic agent in some aggressive forms of periodontitis. The virulence factors of Aa allows it to make contact with host cells, which are potentially important for the occurrence periodontal diseases. In this review we have discussed the various aspect related to leukotoxin secretion, regulation and its mechanism. Understanding the mechanism by which leukotoxin kill the host cell is beneficial in knowing the pathogenesis of the periodontitis.This knowledge may be helpful in diagnosing and treating the periodontitis.

Key words: Aggregatibacter actinomycetemcomitans , leukotoxin , leukocytes ,periodontitis ,virulence factor.

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INTRODUCTION:

Actinobacillus actinomycetemcomitans, a gram-negative coccoid bacillus, is implicated in the etiology of periodontal diseases and also in systemic infection. The prevalence of this bacterium shows a great variation depending on geographic origin and age [1]. A. actinomycetemcomitans is a part of the normal flora but it is also a major agent in aggressive forms of periodontitis [2]. This bacterium has several virulence factors, including production of lipopolysaccharide and cell cycle inhibitors such as a cytolethal distending toxin and a leukotoxin. Both proteins are thought to play a role in immune evasion, but may differ in their target-cell specificity. Leukotoxin is a large pore-forming protein that belongs to the Repeat in Toxin (RTX) family. The leukotoxin selectively affects human cells of hematopoetic origin by

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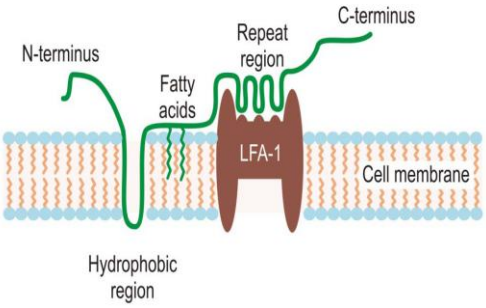
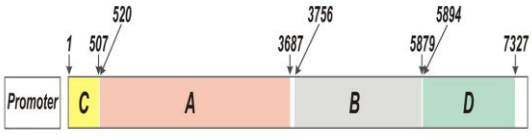
binding to the lymphocyte function associated receptor 1 (LFA-1) and cause disruption of the membrane integrity [3]. It significantly leads to the pathogenesis of periodontal disease.

Structure Of Leukotoxin: Leukotoxin consists of 1055 amino acids encoded by the leukotoxin gene in the leukotoxin operon [4,5]. The molecule can be divided into four regions based upon analysis of the amino acid sequence, the N-terminal region, the central region, the repeat region and the C-terminal region [6].Figure 1

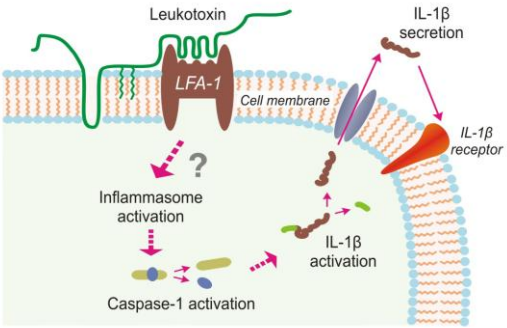


The N-terminal region at residues 1-408 exhibits alternating hydrophobic and hydrophilic clusters. The pore-forming regions of RTX proteins have been suggested to be mediated by the hydrophobic clusters located between residues 175-400 [7]. The central region at residues 409-729 contains large hydrophilic domains and the two acylation sites of leukotoxin located at lysine 562 and lysine 687 [8]. The fatty acids suggested contributing to the anchorage at the target cell membrane. The repeat region consists of tandem repeats of a cassette with nine amino acids located between residues 730-900 and 14 such repeats have been identified in this region of leukotoxin [9]. Finally, residues 901-1055 at the C-terminal end have been shown to be needed for export of the toxin to the bacterial outer membrane by interactions with secretary proteins [10,11]. The four regions of leukotoxin described above are shared among the various toxins in the diverse family of pore forming RTX proteins but their amino acid sequence homology is limited to about 40-50%, with the highest homology between their repeat regions and the lowest between their C-terminal regions [5]. A partial denaturation of the leukotoxin molecule has been reported to enhance its leukotoxicity, which indicates that conformational changes affect the activity of the toxin [12]. leukotoxin crystalline structure has not yet been solved, which limits the available information .

Leukotoxin Expression: The leukotoxin operon consists of four coding genes designated ltxC, ltxA, ltxB and ltxD and an upstream promoter gene [4,5]. The leukotoxin operon is as illustrated in fig. ltxA is encoding for the structure of the toxin, ltxC for components required for posttranslational acylation of the toxin and ltxB and D for transport of the toxin to the bacterial outer membrane. The pattern being similar to the gene organization found for other proteins of the RTX-family [7,13].Figure 2



Interaction with the target cell membrane: Leukotoxin exhibits a unique specificity to cells of haematopoetic origin from humans [14]. The primary role of LtxA is in immune evasion and after it migrates into the periodontal pocket is the infiltration of PMNs to engulf and destroy the bacterium. LFA-1 is a heterodimer composed of 2 proteins, CD11a andCD18, which assemble on the surfaces of essentially all human WBCs. While both molecules are required for LtxA to interact with cells, it was found that CD18 confers species specificity on the toxin [15]. Fatty acids strengthen the anchorage of the toxin when inserted in the target cell membrane. The low concentration of the toxins might induce apoptosis through loss of membrane integrity caused by the small pores. The higher concentration of the toxin allows oligomerization of leukotoxin-LFA-1 complexes on the target cell membrane mediating a rapid and complete membrane collapse [3]. The residues 1-128 on human CD11a has been shown to determine the human specificity of leukotoxin-induced cell lysis [16].Figure 3



It was early shown that human monocytes were as sensitive to leukotoxin, as human PMNs [17]. Killing of monocytes by the toxin proceeds through three distinct phases 1) cessation of the membrane undulating folding and an accumulation of granule in the perinuclear area, 2) abnormal membrane movement and strings of cytoplasm projecting from the cell, and 3) explosive release of cytoplasmic material from the cells [18]. The leukotoxin-induced monocyte lysis involves activation of caspase-1, which indicates involvement of proinflammatory intracellular signalling. Caspase-1 is a cytosolic cysteine proteinase that specifically induces activation and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18) [19,20] . The two cytokines are expressed as biologically inactive precursors and then they are cleaved by caspase-1 for activation and secretion. Caspase-1 is activated by incorporation in a cytosolic multimer complex named the inflammasome [21]. IL-1β is a key component involved in acute and chronic inflammation, which makes the discovery of the leukotoxin induced IL-1β activation relevant and important [22,23]. IL-1 is an important regulator of bone resorption, which associates this cytokine to the alveolar bone loss seen in periodontitis [24,23]. There is a great variation in leukotoxin expression in vitro, although all actinomycetemcomitans strains harbor a complete leukotoxin operon [25].

How leukotoxin secreted: In 2000, it was reported that LtxA was secreted from A. actinomycetemcomitans cells [26]. All strains that were examined to date secrete LtxA, regardless of their serotype or ltx promoter type [27]. A.

actinomycetemcomitans exhibits 2 different leukotoxic phenotypes: minimally leukotoxic (652 strains) and highly leukotoxic (JP2 strains) [28]. Highly leukotoxic strains produced more LtxA protein and ltx mRNA than minimally leukotoxic strains, and an important discovery was the revelation of 2 different types of promoters that control the expression of ltx genes [29]. All 3 proteins—LtxB, LtxD, and TdeA—are required for the secretion of LtxA [30,31].

FACTORS REGULATING LEUKOTOXIN:

IRON:

A unique feature of LtxA is the high specificity for human and Old World primate WBCs [18,32]. It was noted early on that LtxA can target WBCs, but not RBCs [17,33] and A. actinomycetemcomitans is listed as being non-beta-hemolytic [34,35]. However, recently, it was reported that A. actinomycetemcomitans exhibits beta-hemolysis on certain types of growth media [36]. The receptor for RBC is not known and how exactly it is effecting the red blood cells. Because A. actinomycetemcomitans is unable to obtain iron from human transferrin or lactoferrin [37,38] and does not produce siderophores [38,39] . As no free iron is avable in blood and Aa may be using haemoglobin as a iron source. JP2 strains, in contrast to 652 strains, do not produce a functional hemoglobin-binding protein (HgpA) and can thus not utilize hemoglobin as an iron source [37]. Thus, JP2 strains cannot utilize complexed iron from host. so high leukotoxicity (and hence deletion of the promoter region) was strongly selected for in these strains that lacked HgpA, resulting in the JP2 phenotype [40]. There are chances that some could be used by the bacterium if lysis of RBC would result in greater release of heme and haemoglobin. If LtxA plays a role in iron acquisition, one possible scenario is that iron could play a regulatory role in the production of LtxA. Indeed, iron regulates secretion, not transcription, of LtxA [40]. LtxA is essentially fully secreted in iron limiting condition. The mechanism by which iron regulates secretion is not known, but iron may interact with TdeA to cause a decrease in the pore size to prevent the passage of LtxA out of the cell [30].

Role of phosphotransferase system (PTS):

In 2001, [41] reported that the level of fermentable sugars regulates the production of LtxA. Phosphotransferase system (PTS) connects sugars and cAMP. During active transport phosphate is transferred to sugars in cell and for this pts is responsible. Ltx a production can be attenuated by mutation in PTS (ptsH and ptsI). In wild-type strains cAMP levels in these mutants were higher. Wild-type production of LtxA could be restored in the PTS mutants by providing exogenous cAMP in the culture medium. In other bacteria, cAMP is able to regulate gene expression by binding to cAMP receptor protein (CRP), which binds to a cAMP receptor protein binding site on the DNA. A cAMP receptor protein binding site has not been identified in the ltx operon [41], and so whether A. actinomycetemcomitans binds to a

different consensus sequence or acts through a mechanism is not yet clear.

MODIFICATION IN FATTY ACID:

Fatty acid residues are covalently attached to protein counts for unique property of RTX toxins. Fatty acid residues are proposed to play a role in the insertion of toxin into the lipid membrane of the host cell [9,42]. Recently, it was reported that A. actinomycetemcomitans LtxA is also modified at two internal lysine residues; however, the nature of the fatty acids is currently unknown [8]. The modification is not required for secretion as A. actinomycetemcomitans ltxC mutant was able to secrete LtxA at levels similar to the wild-type strain .

ROLE OF COPPER, ZINC AND SUEROXIDE DIMUTASE:

LtxA appears to be stabilized by interaction with another A. actinomycetemcomitans protein, Cu,Zn superoxide dismutase (SOD). during a respiratory burst in host cells, SOD is a bacterial protein that is required for the detoxification of the superoxide radicals that are generated during in this process. SOD interacts with LtxA, and SOD mutants are highly sensitive to superoxide radicals [43]. LtxA protein occurs more rapidly after exposure to reactive , degradation ofLtxA protein occurs more rapidly oxygen species (ROS) and reactive nitrogen species (RNS). It has been reported that LtxA and other RTX toxins induce the production of ROS from host cells [44,45].

LtxA is regulated by other environmental factors, such as pH and oxygen. Lower pH values (6.0-7.0) and anaerobic conditions have positive effects on LtxA production and/or stability [46,47,48] . LtxA is also regulated byquorum-sensing [49] reported that the autoinducer, LuxS, increased production of LtxA. Interestingly, LuxS also increased production of AfuA, a protein involved in iron acquisition [49].

INTERACTION WITH DIFFERENT HOST CELLS:

Polymorphnuclear Cells:

PMNs are the first defense cells to be recruited in the acute phase of an inflammation, as in a periodontal infection [50]. In the infected periodontal pocket, these defense cells are often found at high numbers. They move from the peripheral circulation through chemotaxis towards a gradient of molecules released from as activated host cells. Although PMNs are crucial for phagocytizing and killing bacteria, they also release substances that mediate tissue destruction in aggressive forms of periodontitis [51]. PMNs in the periodontium have been described as a "double-edged sword", capable of producing periodontal disease as well as protecting against the disease [52]. Leukotoxin as well as leukotoxic bacteria have been shown to efficiently cause death of human PMNs and consequently the leukotoxin is assumed to protect A. actinomycetemcomitans against phagocytic killing [25]. The protection occurs in relation to the leukotoxin production of the bacterial population [53].

Further analysis of PMNs exposed to leukotoxin showed an extracellular release of proteolytic enzymes from both primary and secondary granules [54]. Moreover, the interaction between leukotoxin and PMNs mediates activation and release of matrix metalloproteinase 8 [55]. So it can be concluded that leukotoxin before causing death of the PMNs induces activation and release of proteolytic enzymes from these cells. The presence of serum proteins and the relatively high pH (≈8) in the pocket indicates that leukotoxin is released from the bacterial surface in this ecological niche [5,56]. The released toxin is an easy target for inactivation by several of the components present in the periodontal pocket, such as superoxide radicals and proteinases released from the host defense cells or the colonizers of the subgingival biofilm [57,56,43]. In addition, systemic leukotoxin specific antibodies neutralize leukotoxic activity, but if these antibodies are functional in the environment of the infected periodontal pocket is not known [58]. There are also molecules that can protect leukotoxin from inactivation, such as the serum proteinase inhibitors and SOD expressed by A. actinomycetemcomitans [59,43]. As mentioned above, impaired PMN function is closely associated with periodontitis and functional PMNs seem to be of certain importance for combating A. actinomycetemcomitans establishment in the subgingival biofilm [61,51,60,62]. The minimally leukotoxic bacteria are phogocytized and killed by the PMN, while the highly leukotoxic bacteria (JP2) resist PMN phagocytosis and causes extracellular release of lysosomal components [53].

Lymphocytes:

The lymphocytes were initially described as leukotoxin resistant cells [63]. The first observation of leukotoxin susceptible cells of lymphocytic origin was by Simpson and co-workers [32] who showed that several lymphoid cell lines were killed in the presence of leukotoxin. In addition, leukotoxin was shown to suppress function of peripheral blood lymphocytes [64]. A few years later, Mangan and co-workers (1991) showed that T-cells isolated from human peripheral blood were affected by leukotoxin. This leukotoxin-induced T-cell death was a slow process compared to the lysis of human cells of myeloid origin, the death being caused through apoptosis [65] .Human natural killer (NK) cells are affected by leukotoxin in a similar way as the T-cells, while the effects of leukotoxin to human B-cells or plasma cells have not been studied [64]. Human lymphocytes show a great heterogeneity in regard to leukotoxin sensitivity and a subgroup of these cells are shown to be lysed at approximately the same concentrations as human PMNs [66].The variation in leukotoxin sensitivity between PMNs and lymphocytes is not clear. The suggested oligomerization of leukotoxin-LFA-1 complex and the need of lipid rafts on the target cell membrane may indicate that the composition of membrane molecules on the target cells determines then source of leukotoxin-induced death mechanisms

[3]. In cells from the human myeloid carcinoma cell line HL-60, low concentrations of leukotoxin cause apoptosis while higher concentrations lead to necrosis [67]. Cells of lymphoid origin are rare in the infected periodontal pocket but they reside at high numbers in the surrounding tissues as well as in the lymph glands [1]. More than 30 years it has been known that development of periodontitis involves shift from T cell lesion to one involving large numbers of B-cells and plasma cells. A shift in then balance between Th1 and Th2 subsets of T-cells is found in periodontal inflammation, with the Th2 cells to associate with chronic periodontitis [68]. More recently, T regulatory and Th17 cells have been detected in periodontal tissues indicating that these cells also are of importance in the host response and pathogenesis of periodontal disease [69]. The strong humoral immune response induced by leukotoxin indicates direct contact between this molecule and cells of lymphoid origin [58,70]. It may impair the acquired immune response of periodontal infections as leukotoxin has ability to induce apoptosis in lymphocytes.

Erythrocytes: The ability of some strains of A. actinomycetemcomitans to cause β-hemolysis on blood agar plates has been known for many years [71]. Later, it was found that red blood cell lysis caused by A. actinomycetemcomitans involved an interaction with leukotoxin [36]. Different strains of the bacterium with various expressions of leukotoxin show a specific pattern when cultured on blood agar plates containing fresh horse blood. Red blood cells lack the receptor LFA-1, a key molecule for leukotoxin-induced leukocyte lysis [14]. The cellular and molecular mechanisms for the hemolytic effect of leukotoxin are unknown. The lysis of erythrocytes by the leukotoxin has recently been reviewed [72]. So it is difficult to say that whether hemolytic capacity of leukotoxin is an important virulence factor in periodontitis.

CONCLUSION:

The bacteria survive the host immune responses by releasing the ltxa toxin which futher leads death of host cells by creating the favourable environment. Till date there is no animal model for studying the virulence mechanisms of LtxA. In future due to the natural toxicity and specificity of the toxin for certain WBCs, LtxA may show significant therapeutic potential for the treatment of WBC diseases. So it is important to understand the virulence mechanism of ltxa as it not only destroys the defence mechanism of the host but also secretes substances creating favourable environment for bacteria. An association between oral conditions such as periodontal disease and several respiratory conditions has been noted.

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