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Review Article

Pathogenesis of *Aggregatibacter actinomycetemcomitans* in Periodontitis

Ivan Minić and Ana Pejčić

Department of Periodontology and Oral Medicine, Faculty of Medicine, University of Nis, Serbia

***Corresponding Author:** Ivan Minic, Department of Periodontology and Oral Medicine, Faculty of Medicine, University of Nis, Serbia.

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Abstract

Periodontal diseases are widely distributed in the world and represent a major oral health problem both in developed and in developing countries. These chronic inflammatory diseases are characterized by the destruction of tooth supporting tissues. The pathogenesis of periodontal diseases is mediated by the inflammatory response to bacteria in the dental biofilm. More than 700 species have been detected in the oral cavity in different individuals. Approximately 400 of these species have been isolated from different subgingival microenvironments. *Aggregatibacter actinomycetemcomitans* is considered as one of the bacterial species of etiological importance in periodontitis and has contributed to the initiation and/or progression of destructive forms of periodontitis. The potential virulence factors of this organism are powerful leukotoxin, lipopolysaccharide (LPS), cell surface-associated materials, enzymes, and less well-defined virulence factors that will modulate the activity of the host defenses. This organism can induce bone resorption by various virulence factors in periodontal disease.

Keywords: Aggregatibacter actinomycetemcomitans; Periodontitis; Cytokines; Leukotoxin

Introduction

Periodontal diseases are widely distributed in the world and represent a major oral health problem both in developed and in developing countries [1]. These chronic inflammatory diseases are characterized by the destruction of tooth supporting tissues. It is commonly accepted that dental plaque bacteria are the primary etiologic agents of periodontal disease [2]. Knowledge of how immune mechanisms and inflammatory responses are regulated is critical for understanding the pathogenesis of complex diseases, such as periodontitis [3]. The pathogenesis of periodontal diseases is mediated by the inflammatory response to bacteria in the dental biofilm. More than 700 species have been detected in the oral cavity in different individuals. Approximately 400 of these species have been isolated from different subgingival microenvironments [4]. However, only a few species have been associated with the disease. From these bacteria specifically associated with destructive disease, *Aggregatibacter actinomycetemcomitans* is considered as one of the bacterial species of etiological importance in periodontitis and has contributed to the initiation and/or progression of destructive forms of periodontitis [5].

Aggregatibacter actinomycetemcomitans (previously *Actinobacillus actinomycetemcomitans*) is a Gram-negative, facultative anaerobe, non-motile bacterium that is often found in association with localized aggressive periodontitis, a severe infection of the periodontium [6]. It is also suspected to be involved in chronic periodontitis. *A. actinomycetemcomitans* commonly is part of the normal flora of the mouth of humans, especially the gingival and supragingival crevices, and also can be found in various animal species [7]. The incidence of carriage is markedly higher in individuals with refractory periodontitis and in people with juvenile periodontal disease. The organism may be transmitted among household members [8].

The most common illness associated with *A. actinomycetemcomitans* is juvenile periodontal disease. *A. actinomycetemcomitans* also causes soft-tissue abscesses, particularly of the chest wall or mandibular area, often as a coinfection with *A. israelii*. Chest wall lesions often are associated with pulmonary or pleural lesions and lesions of the mandibular area typically are associated with dental caries or periodontal disease. Cases of pneumonia, empyema, osteomyelitis, septic arthritis, brain abscess, cervical lymphadenitis, intra-abdominal abscess, and urinary tract infection have been reported with *A. actinomycetemcomitans* as a sole pathogen or copathogen [9,10].

A. actinomycetemcomitans best grow at a temperature of 37°C in the presence of 5% CO_2 while the optimum pH ranges in the range of 7.0 and 8.0 [11]. In the liquid medium, the organism forms isolated, translucent granules which adhering to the walls or bottom of the tube, and the medium remains clean. On blood agar and selective TSBV substrate [12] creates small, convex, translucent, Circular colonies, diameters about 1 mm after 2 - 3 days. Colonies have discretion irregular edges and very firmly attached to the surface of the agar. The rough surfaces are when viewed under a light microscope, a center is in the shape of a star or the appearance of crossed cigarettes. Isolates *A. actinomycetemcomitans* are classified into six serotypes (a-f) [13]. The serological specificity determines the presence of O-polysaccharide, a large molecule molecular weight, which in addition to lipids enter the lipopolysaccharide-LPS (SPAs-serotype specific polysaccharide antigen) [14] *A. actinomycetemcomitans* colonization are due to individual immune responses, a heterogeneity in *A. actinomycetemcomitans* or both. Currently, seven different *A. actinomycetemcomitans* strain serotypes have been identified, differing by O-polysaccharide (O-PS) structures of lipopolysaccharides, though *A. actinomycetemcomitans* heterogeneity relies on more than just O-PS gene clusters [15].

Investigated *A. actinomycetemcomitans* gene expression and protein expression at the transcriptional and translational levels in human serum.

Roe., *et al.* are 2002. sequenced the *A. actinomycetemcomitans* gene ATCC 700685 (HK1651, JP2 clone) at the University of Oklahoma [16]. Genome consists of 2,024,943 bp and the current sequence at this stage represents 99.8% genome. The chromosome is represented by a single circular molecule and is the largest similar to chromosome *H. influenzae* Rd [1,830,137 bp], which is considered to be the closest cousin *A. actinomycetemcomitans*. Overall, the authors found that particular strains of *A. actinomycetemcomitans* responded specifically to human serum with a second rapid increase of turbidity in the serum culture broth [17]. They suggest this is a consequence of cell deterioration and protein aggregate formation and is most likely triggered by interactions between human serum and the bacterial membrane or parts thereof. In addition, they suggest that this activates an extracytoplasmic stress response controlled by rpoE, which then causes different accessory genes to be activated, resulting in high- and low-responder strains, which impacts *A. actinomycetemcomitans* pathogenesis [18].

Cytokines induced by *A. actinomycetemcomitans*

Research done for the purpose of revealing immunomodulatory activity *A. actinomycetemcomitans* bacteria have come up with results that indicate very unusual host response to the presence of this bacterium. Exposure to human the fibroblasts in this bacterium induced the synthesis of IL-6 and IL-8, but not IL-1β [19]. Cytokines are protein-nature molecules that bind to cell surface receptors activating cell proliferation and/or differentiation, activation and apoptosis mechanisms.

The cells that secrete the largest amounts of cytokines are called leukocytes. Cytokines secreted by lymphocytes are called lymphokines, monocyte and macrophage cytokines are called monokines, while many lymphokines are known as interleukins [20].

Pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) have been distinguished as inflammatory mediators whose production intensity is an indicator of the periodontium tissue destruction activity. Patients with periodontal disease have been shown to have elevated values of these pro-inflammatory cytokines in the gingival fluid compared to respondents with healthy periodontal tissue [21,22].

Due to the increased permeability of periodontium blood vessels, which occurs as a result of inflammation, pro-inflammatory cytokines have the ability to penetrate the systemic circulation.

Studies have shown that intact *A. actinomycetemcomitans* stimulates humanity mononuclear cells to produce chemokines MIP-1α (macrophage inflammatory protein) and RANTES (regulated on activation, normal T cell expressed and secretion) [23]. Recent advances in cellular and molecular biology have allowed investigators to better understand the mechanisms of inflammatory and immune responses in many infectious diseases. Soluble mediators produced by various inflammatory and structural cells, collectively called cytokines, have been shown to play a crucial role in the pathogenesis of most of these diseases, including periodontal disease. This paper globally reviews recently reported findings implicating cytokines in periodontal pathophysiology [24].

Macrophage Inflammatory Proteins (MIP) belong to the family of chemotactic cytokines known as chemokines. In humans, there are two major forms, MIP-1α and MIP-1β that are now officially named CCL3 and CCL4, respectively.

Both are major factors produced by macrophages after they are stimulated with bacterial endotoxin. They are crucial for immune responses towards infection and inflammation. They activate human granulocytes (neutrophils, eosinophils and basophils) which can lead to acute neutrophilic inflammation. They also induce the synthesis and release of other pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6 and TNF-α from fibroblasts and macrophages. The genes for CCL3 and CCL4 are both located on human chromosome 17 [25,26].

A. actinomycetemcomitans and immunosuppression

A. actinomycetemcomitans produces two such immunomodulatory toxins leucotoxin and cytolytic toxin stretching. Leukotoxin is a member of the highly conserved repeat toxin (RTX) family of bacterial toxins expressed by a variety of pathogenic bacteria. While the RTX toxins of other bacterial species are secreted, the leukotoxin of *A. actinomycetemcomitans* is thought to remain associated with the bacterial cell. This group of toxins, forming channels on the cell membrane, leads to the death of the cell by an osmotic lysis (high doses) or induction of apoptosis (lower doses, probably more representative in physiological conditions). RTX toxins consist of a specific sequence of amino acids that is repeated more than 40 times [27].

Two major strains of *A. actinomycetemcomitans* have been reported, a minimal leukotoxin producing strain (652 type) and hyper-producing leukotoxin strain (JP2 type). At the genetic level the hyper-producing strain shows a deletion of 530 bp in the promoter region that appears to be responsible for increased expression of downstream ltx genes.

Regulation of ltxA expression has been studied extensively at the molecular level in naturally occurring promoter-deleted strains described as JP2-like strains [28].

Studies *in vitro* have shown that low concentration of LtxA promotes neutrophil degranulation, releases collagenolytic proteinase - matrix metalloproteinase 8 (MMP 8) [29] and inhibited phagocytosis [30]. These effects can be the result of an increase in the concentration of Ca_2^+ intracellularly, which occurs under the influence of LtxA [31]. High concentrations of toxins in *in vitro* conditions can led to a lysis of the cell. LtxA causes degeneration of lysosomes results in damage to the host tissue and triggers an inflammatory response. Five genes are required for successful translation and secretion of LtxA (the structural toxin gene product) in *A. actinomycetemcomitans*. Four of the genes, ltxCABD (in transcriptional order) are located in the leukotoxin operon. A fifth gene, tdeA (6), is not part of the ltx operon and is located 572 kb downstream of this site. Prior to secretion, Gram-negative bacterial protein toxins are translated as protoxins and must be post-translationally modified to achieve biological activity.

This modification takes various forms with bacterial protein toxins. In the case of RTX toxins, the RTXC protein, an acylase, catalyzes the attachment of fatty acyl chains to internal lysine residues of the toxin in the bacterial cytoplasm; this acylation process is necessary for the toxin to achieve its biological activity [32]. The remaining three gene products (LtxB, LtxD, and TdeA) form a type I secretion system and export LtxA directly from the bacterial cytoplasm to the external environment without the need for a periplasmic intermediate [33].

Other immunomodulators/inhibitors of the cell cycle

There is a whole series of insufficiently characterized immunomodulators/inhibitors of the cell cycles produced by *A. actinomycetemcomitans*.

This group also includes protein, from 14 kDa, which is purified and claimed to inhibit the synthesis of lymphokines (IL-2, IL-4) [34]. Protein Omp34, which is a part of the external membrane bacteria *A. actinomycetemcomitans*, functions as Fc binding protein and thus demonstrates its immunosuppressive properties fact.

It has also been reported that this organism produces a molecule of small molecular weight mass that inhibits neutrophil chemotaxis [35].

Conclusions

The ability of LtxA to cause death of all subsets of cells with hematopoietic origin might contribute to help the bacterium to survive the host immune response and also to release compounds essential for bacterial growth. The more recent discoveries that LtxA mediates activation and release of proteolytic enzymes from PMNs and proinflammatory cytokines from monocytes/ macrophages indicate a more direct role of LtxA in the pathogenesis of periodontal diseases.

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