Biomedical & Pharmacology Journal Vol. **8**(Spl. Edn.), 249-252 (Oct. 2015)

Aggregatibacter Actinomycetemcomitans: its Role in Periodontitis

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DOI: http://dx.doi.org/10.13005/bpj/685

(Received: July 25, 2015; accepted: September 10, 2015)

ABSTRACT

Strong evidence is available on the role of Aggregatibacteractinomycetemcomitans (A.a) as the causative agent of localised juvenile periodontitis(LJP), a disease characterised by rapid destruction of the tooth-supporting tissues. This organism possesses a large number of virulence factors with a wide range of activities which enable it to colonise the oral cavity, invade periodontal tissues, evade host defences, initiate connective tissue destruction and interfere with tissue repair. Adhesion to epithelial and tooth surfaces is dependent on the presence of surface proteins and structures such as microvesicles and fimbriae. Invasion has been demonstrated in vivo and in vitro. The organism has a number of means of evading host defences, which include: (i) production of leukotoxin; (ii) producing immunosuppressive factors; (iv) secreting proteases capable of cleaving IgG; and (v) producing Fc-binding.

Key words: Periodontitis, Leukotoxin, Lipopolysaccharide.

INTRODUCTION

It has been well documented that biofilm bacteria predominate, numerically and metabolically, in virtually all nutrient-sufficient ecosystems, including the oral cavity[1,2]. Bacterial cells in biofilms are surrounded by a self-synthesized, three-dimensional matrix, which holds the cells together and firmly attaches the bacterial cells to the underlying surface[3]. The extracellular polymeric substance has been attributed to a protective role as well as it is a source of dissolved nutrients, secreted enzymes, extracellular DNA and exopolysaccharide. The exopolysaccharide of Aggregatibacteractinomycetemcomitans (PGA) is a homopolymer of N-acetyl-D-glucosamine residues in â(1,6) linkage and has been well characterized in several bacteria including Staphylococcus aureus, S. epidermidis and E. coli. This exopolysaccharide has been named differently in various bacteria but its synthesis is encoded by a set of four genes, icaADBC in Staphylococcal species and pgaABCD in E. coli and A. actinomycetemcomitans. The exopolysaccharide from A. actinomycetemcomitans, PGA, is a surface-associated polymer that can protect A. actinomycetemcomitans at the cellular level from phagocytic killing[4]. A similar protective function was ascribed to the exopolysaccharide PIA of S. epidermidis[5]. PGA/PIA mediates resistance to killing by antibiotics[6], detergents[7] and antimicrobial peptides[5]. PGA may act through a general mechanism wherein it binds to or electrostatically repulses immune modulators and antimicrobial agents, thereby preventing their access to the bacterial cell[5]. The importance of PGA in a protective role for A. actinomycetemcomitans and other bacteria has been well established[4]. In addition, a recent study of Ps1, the exopolysaccharide of P. aeruginosa, has demonstrated that it also determines the fate of elite cells in the initial microcolony development[8]. While these studies highlight the significance of exopolysaccharide, they also bring the genes encoding the exopolysaccharide to the forefront in the disease process. For example, in several infection models, the exopolysaccharide PIA has been demonstrated to be relevant for the virulence of S. epidermidis[9,10,11].

Morphology

Aggregatibacteractinomycetemcomitans (actis, a ray; myces, a fungus; comitans, accompanying; actinomycetemcomitans, accompanying an actinomycete) is a gram-negative coccobacillus measuring about 0.4 ± 0.1X 0.1 ± 0.4 micrometers in size. Aggregatibacter actinomycetemcomitans possess fimbriae, vesicles and extracellular amorphous materials. MGB (trypticase soy broth) with malachite green and bacitracin was the earliest media used to culture (A.a). It was then followed by medium with trypticase soy agar, serum with bacitracin and vancomycin (TSBV). Exclusive growth of A.a was found in a particular culture medium which contained TSBV, spiramycin, fucidic acid and carbencillin. RPMI – 1640 and Dulbecco's modified Eagle medium are now used with a generation time of 246 and 346 min[12].

Virulence factors

The putative virulence factors of A. actinomycetemcomitans can be subdivided into those that: (i) modulate inflammation, (ii) induce tissue destruction and (iii) inhibit tissue repair. The most actively studied gene product of the organism is a leukotoxin and a member of the RTX (repeats in toxin) family whose cellular receptor is the integrin, LFA-1, thus accounting for its selective effect on leucocytes (although only those from primates)[13-15]. Almost all the RTX leukotoxins are secreted except LtxA toxin of A. actinomycete mcomitans which is thought to be entirely cell associated; either bound to cell surface-associated nucleic acids[16] or within membranous vesicles which bud from bacterium's surface[17,18]. This affirms to the possibility that the bacterium itself is toxic to the target cells. The apoptosis of the target cells in response to A.actinomycetemcomitansleuko toxin is by a mechanism involving mitochondrial perturbation[19]. Injection of A. actinomycete mcomitans into mice has been claimed to induce immunosuppression and sonicates of this organism suppressed the IgG response to sheep red blood cells in mice[20,21]. It has also been proposed that A.actinomycetemcomitans can produce super antigens, which have the ability to bring about T cell apoptosis by binding to T cell receptors[22,23]. A. actinomycetemcomitans has been reported to produce a number of, as yet unidentified, proteins with cell cycle-inhibitory activity causing arrest in the G2 phase of the cell cycle. These proteins range in molecular mass from the 8-kDa protein termed gapstatin to 60 kDa and all the way up to 80 kDa[24]. One cell cycle-modulatory protein with immunosuppressive function that has recently been identified as being produced by A. actinomycetemcomitans is cytolethal distending toxin (CDT) Fc binding protein terme as Omp34 identical with OmpA of E. coli, a protein implicated in the virulence of this organism is another immunomodulatory virulence factor of A. actinomycemcomitans. A. actinomycetemcomitans produces a 65-kDa macromolecule able to bind to the IL-10 receptor and henceforth can modulate monocyte/macrophage function as IL- 10 is considered to be a major macrophage de-activating cytokine.A. actinomycetemcomitans has also been reported to produce a low molecular mass inhibitor of neutrophil chemotaxis to FMLP. LPS is reported to stimulate bone resorption in vitro and in vivo. But it is considered to be a less significant cytokine inducer than the secreted protein. A cell stress protein, chaperonin 60 is considered to be a potent bone degrading molecule bystimulating bone resorption by acting as an osteoclast 'growth factor'

Virulence mechanism

Adhesion

Bacterial adhesion, which facilitates colonization is the key virulent mechanism[25] Bacterial components involved in Adhesion are called adhesins. They are proteinaceous structures found on cell surfaces. They bind with specific receptors in the saliva, tooth, extra cellular matrix and epithelial cells. Surface entities like vesicles mediate aggregation. A.actinomycetemcomitans adheres to the gingival crevice epithelium. Strains with fimbriae adhere three to four folds better. A.actinomyc etemcomitans binds to collagen I,II,III and V but not IV. It also binds to fibronectin but not fibrinogen. The tight auto-adhesion of A. actinomycetemcomitans has been described is due to the expression of long, bundled fibrils composed of a 6.5-kDa subunit protein,Flp-1 (fimbrial low-mol. wt protein) which has been reported to be glycosylated .Bacteriocins are proteins produced by bacteria that are lethal for other strains and species of bacteria. These agents confer colonization by lessening ecological pressures. This is an advantage for the bacterium.

Invasion

It has been affirmed that many bacteria have the ability to invade host cells and A. actinomycetemcomitans is one among them. Studies of invasion of A. actinomycetemcomitans reveal that 25% of A.actinomycetemcomitans isolates are invasive. A. Actinomycetemcomitans penetrate and survive within eukaryotic cells. They penetrate gingival epithelium. They occur in specific intracellular locations like the epithelial wall, enlarged intracellular pocket spaces and the epithelial side of basal lamina in connective tissue and alveolar bone. It has been observed that microfilaments and microtubules for intracellular movement . The process of intracellular movement and the cell spreading could be inhibited by agents that interfered with microtubule dynamics, suggesting that this bacterium when internalized interacts closely with the microtubules of the host cell . It has been suggested that the transferrin andd integrin receptors are involved in the adhesion of the bacteria to host cells .

CONCLUSION

A.actinomycetemcomitans is a highly non motile gram negative coccobacillus with a vast array of potential virulence factors and mechanisms. Though it was initially named as Actinobacillusactinomycetemcomitans, it was found that the bacterium is more similar to haemophilus than actinobacillus and hence it was reclassified under aggregatibacter as Aggregatibacteractinomycetemcomitans. Scientific data clearly underlines its etiological role in localized aggressive periodontitis. This review also tries to throw light on the virulence abilities of this pathogen like immune evasion mechanisms like production of leukotoxin, cell cycle modulatory protein and immunomodulatory protein like Fc binding proteins. It also brings about tissue destruction by other novel mechanisms like binding to host matrices and invading host cells. Still, a lot is still to be understood and established. With the advent of newer technological methodologies and genome information, we would be able to understand not only how A. actinomycetemcomitans produces such profound but local pathology like periodontal infections but also its role in systemic pathology

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