Aggregatibacter actinomycetemcomitans: Virulence of its leukotoxin and association with aggressive periodontitis

Carola Höglund Åberg[1], Peyman Kelk[2], and Anders Johansson[1,*]

[1]Division of Molecular Periodontology; Department of Odontology; Faculty of Medicine; Umeå University; Umeå, Sweden; [2]Section for Anatomy; Department of Integrative Medical Biology; Faculty of Medicine; Umeå University; Umeå, Sweden

Periodontitis is an infection-induced inflammatory disease that causes loss of the tooth supporting tissues. Much focus has been put on comparison of the microbial biofilm in the healthy periodontium with the diseased one. The information arising from such studies is limited due to difficulties to compare the microbial composition in these two completely different ecological niches. A few longitudinal studies have contributed with information that makes it possible to predict which individuals who might have an increased risk of developing aggressive forms of periodontitis, and the predictors are either microbial or/and host-derived factors. The most conspicuous condition that is associated with disease risk is the presence of *Aggregatibacter actinomycetemcomitans* at the individual level. This Gram-negative bacterium has a great genetic variation with a number of virulence factors. In this review we focus in particular on the leukotoxin that, based on resent knowledge, might be one of the most important virulence factors of *A. actinomycetemcomitans*.

Periodontal Diseases

Inflammation is an essential immune response that enables survival during infection or injury and maintains tissue homeostasis under a variety of noxious conditions.[1] The type of pathway induced under a given condition depends on the nature of the inflammatory stimuli. Thus, bacterial pathogens are detected by receptors of the innate immune system on the tissue-resident macrophages, and those cells induce production, activation and secretion of inflammatory mediators.[1] These mediators induce vasodilation, neutrophil recruitment,

and leakage of plasma into the infected tissue. The neutrophils recruited from the circulation, the tissue-resident macrophages, and the mast cells seek and destroy invading pathogens. Microbial pathogens located in the oral biofilm can contribute to the pathogenesis of periodontal diseases by releasing components that cause imbalance in the host inflammatory response.[2] Periodontitis is a bacteria-induced inflammatory disease that causes loss of the tooth-supporting tissues, alveolar bone and connective tissues.[3] The disease is highly prevalent and affects billions of people around the world. Inflammatory periodontal diseases generally progress slowly, but may at any stage undergo exacerbation resulting in additional loss of the tooth attachment and subsequently tooth loss. Various host genetic factors have been shown to interfere with the subgingival oral biofilm, a phenomenon named periodontal infectogenomics.[4] In addition, there is a significant association between periodontitis and an increased risk for systemic diseases, such as arteriosclerosis and endocarditis.[3,5,6]

The periodontal infection is polymicrobial and has been shown to contain hundreds of different bacterial species in the same periodontal lesion.[7] Decades of investigations have demonstrated that the microbial communities associated with periodontitis differ from those in health.[8] Microbiome science continuously accumulates huge amounts of information about complex microbial communities, and that knowledge needs a healthy dose of skepticism.[9] The causality of the microbiome pattern in relation to disease initiation and progression is still not fully understood. The contributions of specific bacteria to disease may be unimportant according to the ecological plaque hypothesis.[10] Targeting one or more "pathogens" will not necessarily cure disease, since other organisms with similar activities might take their place.[2] Therefore, it may make more sense to focus on the specific virulence factors that contribute to disease, rather than on the microorganisms that produce those factors.[7] However, a subgroup of periodontitis includes the aggressive form that affects young individuals and has a high rate of periodontal disease progression. The aggressive forms of the disease are in most cases correlated to the presence of certain bacterial species in the subgingival biofilm with a predominance of

Gram-negative anaerobic rods that colonize the periodontal crevice.[11] The contribution of specific pathogenic bacteria to disease implies an infection with a highly virulent microflora that releases factors, which can cause an imbalance in the host response.[2]

The rapidly-progressing form of periodontal disease, named Aggressive periodontitis (AgP), affects young otherwise healthy individuals. The disease process is initiated by the microbial challenge that induces an aggressive inflammatory response, and the process results in a periodontal destruction in the tooth-supporting soft tissues and alveolar bone[12] (Fig. 1). If the inflammatory trigger is not eliminated by the acute inflammatory response or persists in the subgingival biofilm in the periodontal crevice, severe tissue damage may occur. The resulting tooth loss at an early age may have profound cosmetic, functional, and psychological effects.[13] It might be difficult to convince the observer that the bacterial burden in these cases can initiate an inflammatory response that may result in severe tooth loss, since the initial clinical presentation frequently shows little visible plaque accumulation.[14,15]

Two forms of AgP, the localized form (LAgP), and the generalized form (GAgP), have been distinguished based on the number of teeth affected and the distribution of the lesions within the dentition. For a long time, aggregation within

families has been linked to aggressive forms of periodontitis and may be explained by a genetic predisposition related to susceptibility to the disease. Ethnicity and sociodemographic status have been shown to affect the carriage patterns of periodontal pathogens.[11,16,17] Childhood and adolescence are critical time points for the onset of AgP. The two main diagnostic criteria used for the detection of periodontitis are the loss of connective tissue attachment and loss of alveolar bone based on clinical and radiographic assessments. The fact that AgP, especially the localized form (LAgP), is associated with the presence of *Aggregatibacter actinomycetemcomitans*, is well known.[18] Data concerning this linkage are mainly based on association studies, while causality is difficult to determine due to the complexity of the disease.

Aggregatibacter actinomycetemcomitans

The Gram-negative *A. actinomycetemcomitans* is assumed to be the primary etiologic agent of LAgP and has also been implicated in chronic periodontitis and severe non-oral infections.[18] Currently seven serotypes of this bacterium (a-g) are recognized based on the immunodominant antigen, which is an O-polysaccharide of the lipopolysaccharide (LPS).[19,20]

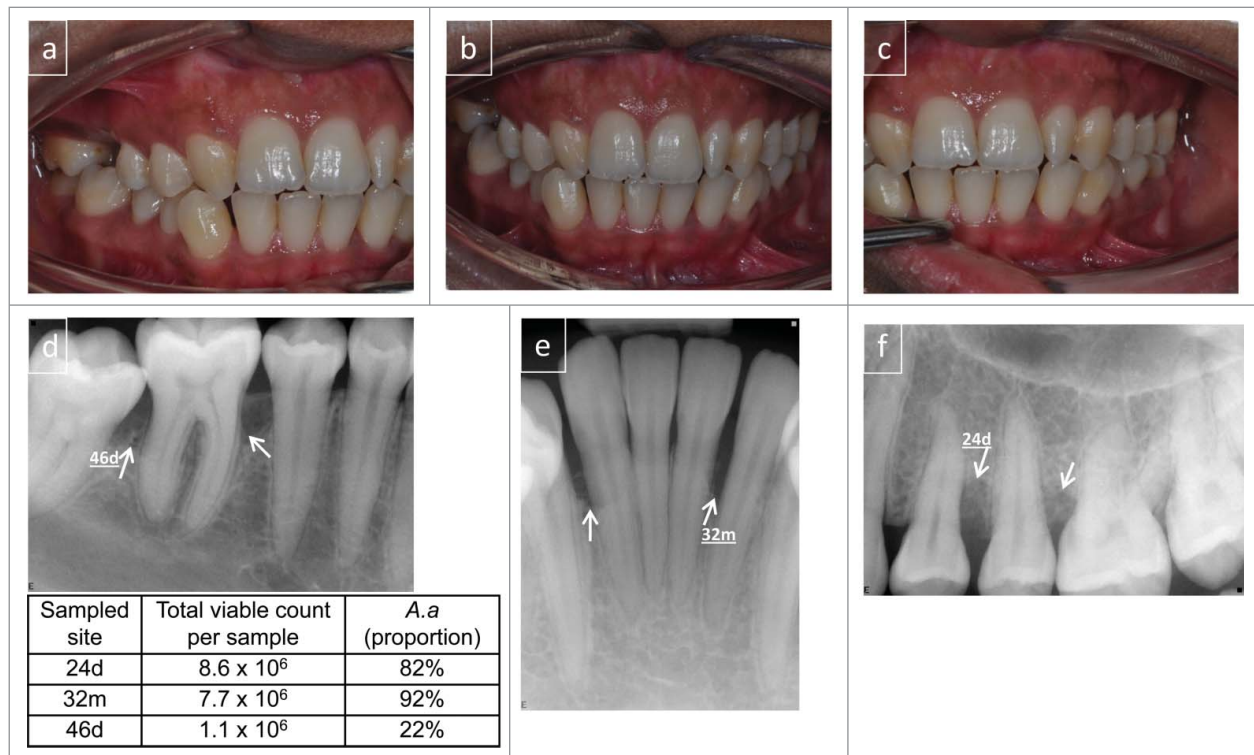| Sampled site	Total viable count per sample	*A.a* (proportion)
24d	8.6×10^6	82%
32m	7.7×10^6	92%
46d	1.1×10^6	22%

Figure 1. Clinical presentation of localized aggressive periodontitis. A 16-year old female presenting with radiographic alveolar bone loss associated with bone defects (marked with arrows) and probing attachment loss at 2 permanent first molars, left upper premolars and lower incisors. Clinical photographs buccal view (**A–C**). Radiographs (**D–F**). The clinical presentation shows sparse plaque accumulation and localized gingival inflammation with 4–8 mm periodontal crevices with bleeding on probing in the affected regions. The results from the microbial sampling are presented in the inserted table and the sampled sites are indicated. Microbiological analysis, by cultivation technique, confirmed the presence of high levels of *A. actinomycetemcomitans* in the 3 lesions sampled. Diagnosis: localized aggressive periodontitis (LAgP).

This bacterium has a complex lifecycle, acquired through transmission from saliva of infected individuals, and may initially colonize the oral mucosa possibly as a facultative intracellular pathogen.[11] The bacterium moves from the initial oral colonization site to the gingival crevices and competes with other bacteria in the niche. Successful establishment of persistent colonization in subgingival crevices by *A. actinomycetemcomitans* may lead to periodontal destruction and development of periodontitis in susceptible individuals.[21] Fine and co-workers[22] reported that soft tissue binding is mediated by the adhesins ApiA and Aae that bind specifically to the buccal epithelium. *In vitro* experiments show that the bacteria can be translocated from the epithelium to the hard surfaces (hydroxyapatite, enamel) but not in the opposite directions.[22] These data support the argument that *A. actinomycetemcomitans* may use buccal cells as a reservoir for initial attachment before the bacteria eventually move to the non-shedding tooth surfaces.

The virulence potential of *A. actinomycetemcomitans* appears to vary among strains, and specific serotypes/clonal types of the bacterium have been reported to be more prevalent in individuals with aggressive forms of the disease.[18] Prospective population-based studies have shown that individuals infected by strains of the serotype b JP2 genotype of *A. actinomycetemcomitans* have a significantly higher risk of developing AgP than individuals infected by strains of the non-JP2 genotype.[17,23] The JP2 genotype is defined by a 530-bp deletion in the promoter region of the leukotoxin operon, and this genotype is highly leukotoxic.[24] The within-species variable virulence of *A. actinomycetemcomitans* may be attributed to strain-to-strain variation in the genome content and in the regulation of virulence gene expressions.[20]

The ability of the bacterium to express a leukotoxin (LtxA) is considered to be an important virulence property.[18] The toxin kills white blood cells in a variety of ways, and leukocyte destruction is essential for subsequent bacterial growth and stimulation of the host inflammatory response.[25] Leukotoxicity is substantially correlated with attachment loss in adolescents, indicating an important role of the toxin in the pathogenesis of AgP.[26] Among the non-oral infections, pneumonia caused by *A. actinomycetemcomitans* infections dominates of reports from adolescents.[27] There was an association to periodontitis in these *A. actinomycetemcomitans* infected individuals, but the virulence characteristic of these bacteria has not yet been examined. It has been demonstrated that members of the HACEK group (*Haemophilus* and *Aggregatibacter* spp., *A. actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae*), especially *A. actinomycetemcomitans*, are associated with systemic diseases far from the oral cavity.[28,29] The great genetic diversity within this species indicates that the highly virulent genotypes might be associated with the non-oral infections.

A. actinomycetemcomitans LtxA is a large pore-forming toxin that belongs to the RTX (Repeats-in-toxin) family of bacterial proteins.[30] There is great variation in LtxA expression *in vitro*, although all *A. actinomycetemcomitans* strains harbor a complete *ltxA* operon.[20] Expression of LtxA is not fully characterized, but

it has been shown that the expression is regulated by both environmental and genetic factors.[25]

Virulence Mechanisms of *A. actinomycetemcomitans*

The genetic diversity among different isolates of *A. actinomycetemcomitans* interferes with the ability of the bacterium to express and release virulence factors.[20] The different adhesins and fimbriae expressed by this bacterium have been shown to be important factors that promote colonization at the various ecological niches of the human oral cavity.[22] The periodontal infection by *A. actinomycetemcomitans* is accompanied by local and systemic immune responses, and this species may invade the gingival epithelium and release virulence factors such as endotoxins and exotoxins.[18] Endotoxin is expressed by all Gram-negative bacterial species and causes a general pro-inflammatory host response, while the 2 exotoxins, a cytolethal distending toxin (Cdt) and a leukotoxin (LtxA), are unique for *A. actinomycetemcomitans* within bacterial species colonizing the oral biofilm.[18] Cdt's are expressed by a number of Gram-negative bacteria and cause death of the host cells by blocking their proliferation and enhancing the expression of the Receptor activator of nuclear factor kappa-B ligand (RANKL), a key factor in osteoclastogenesis.[31,32] Despite the pathogenic potential of the Cdt *in vitro*, a recent longitudinal study performed on adolescents in Ghana could not demonstrate any significant differences in disease progression between individuals colonized with Cdt-expressing *A. actinomycetemcomitans* and individuals colonized with bacteria that lack Cdt expression.[33] The LtxA selectively affects human cells of haematopoietic origin by binding to the lymphocyte function-associated receptor 1 (LFA-1) and causes disruption of the membrane integrity.[34] Apart from causing death of the defense cells, LtxA also induces a massive pro-inflammatory response in human monocytes/macrophages.[35] There is a strong association between the presence of highly leukotoxic *A. actinomycetemcomitans*, both of the JP2-genotype and some highly leukotoxic variants of the non-JP2 genotype, and the disease progression in the infected individuals studied.[17,23,26] These findings attract enhanced attention concerning the importance of leukotoxicity acting toward the defense mechanisms in the host response.

Leukotoxin-Induced Pro-Inflammatory Cell Death

A. actinomycetemcomitans LtxA belongs to the RTX-toxin family and shares substantial molecular homology with other toxins of the RTX family such as *Escherichia coli* α-hemolysin and *Mannheimia haemolytica* leukotoxin.[30] RTX toxins are divided into 2 categories based on their target cell specificity. RTX hemolysins, such as *E. coli* α-hemolysin (HlyA) and *Actinobacillus pleuropneumoniae* ApxIA, are toxic to a wide range of cell types from many species. In contrast, leukotoxins of *A. actinomycetemcomitans*
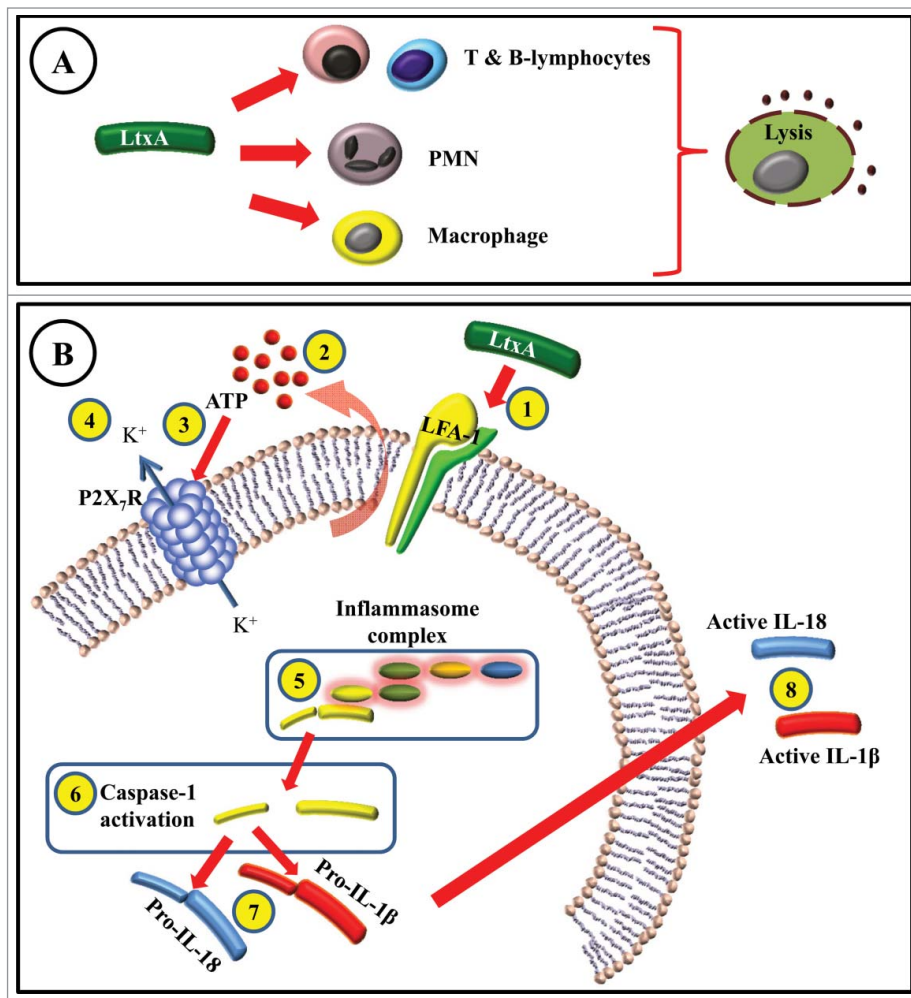
Figure 2. (**A**) Interaction of *A. actinomycetemcomitans* leukotoxin (LtxA) with T-, B-lymphocytes, polymorphonuclear (PMN) leukocytes, and macrophages leads to cell lysis. (**B**) LtxA-induced cell death in macrophages involves several steps before the cell lysis occurs. LtxA binds to LFA-1 (**1**) and induces extracellular release of ATP (**2**). The released ATP binds to the P2X$_7$-receptor (**3**) that subsequently causes efflux of potassium (**4**). The inflammasome complex is formed and activated (**5**), which promotes the cleavage and activation of a cysteine proteinase called caspase-1 (**6**). The cleaved caspase-1 is then responsible for activation (**7**) and release of abundant levels of active IL-1β and IL-18 (**8**).

pathological conditions by promoting osteoclast survival and activation, although IL-1-mediated osteoclastogenesis requires the receptor activator of NF-kappaB ligand (RANKL).[41] Production of pro-inflammatory cytokines, including TNF-α, and IL-1β, by monocytes/macrophages in response to extracts and components of *A. actinomycetemcomitans*, has in fact been shown.[18] However, the involvement of LtxA in these cell activations has not been investigated in the 20th century, probably because LtxA has mainly been considered to disrupt the host defense system by killing the immune cells. With this in mind, we postulated that perhaps LtxA itself could not only be responsible for killing of the cells, but also play a major role in the induction of inflammatory responses of importance for the pathogenicity in periodontitis. Our in depth mechanistic studies of LtxA's effects on macrophages have revealed intriguing findings. We have shown for the first time that macrophages are the most sensitive cells to LtxA-induced cell lysis and that this process consequently causes a specific pro-inflammatory cell death. The LtxA-induced macrophage lysis also involves a specific activation of caspase-1 that subsequently leads to excessive secretion of IL-1β from the macrophages.[35,42] Since LtxA is primate-specific,[18] conducting experiments on animals is of little biological interest. Therefore, we used an alternative to test the already secreted IL-1β from LtxA-challenged human macrophages in an animal bone resorption model. The secreted IL-1β, from LtxA-exposed macrophages, was mainly the biologically active form and could act as the major activator of bone resorption in the tested bioactivity assay.[42] To investigate the contribution of LtxA in relation to other bacterial products from *A. actinomycetemcomitans*, macrophages were exposed to live strains of *A. actinomycetemcomitans*. In this study we were able to show that indeed the rapidly induced IL-1β secretion from the macrophages was mainly due to the LtxA.[43]

There are major steps before the inactive form of this protein is activated and the active IL-1β is secreted. A crucial step is the activation of caspase-1 that occurs in specialized caspase-1 multiprotein complexes referred to as inflammasomes.[44] The investigation of mechanisms involved in the caspase-1 inflammasome-activation is now a "hot" field in cytokine research. There are numerous pathways for activation of the caspase-1 inflammasome by pathogen-associated molecular patterns (PAMPs) and

(LtxA) and *M. haemolytica* (LktA), are only toxic to specific types of cells in specific species.[34] It has long been known that LtxA selectively kills human leukocytes (**Fig. 2A**) and disturbs the local defense mechanisms.[25,36]

One of the most important pathogenic mechanisms involves the production of cytokines, which stimulate inflammatory events that induce other effector mechanisms. Regarding this aspect, macrophages are one of the major sources of pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF).[37] These cytokines improve the activation of cellular immunity and intensify the inflammatory cascade. However, when there is an imbalance in the host response, these cytokines can be harmful for the host tissues.[38,39] One such impaired immune response is the case of periodontitis, where osteoclast-differentiation is closely associated with the differentiation of macrophages.[40] IL-1 is involved in bone loss in various

danger associated molecular patterns (DAMPs). The inflammasones respond to various molecules such as extracellular adenosine triphosphate (ATP), different crystals (monosodium urate (MSU)), calcium pyrophosphate dihydrate (CPPD), silica, asbestos, aluminum salts, and ameloid-β), bacterial products (muramyl dipeptide (MDP) and flagellin), and toxins (Nigericin, Maitotoxin and aerolysin).[45,46] Furthermore, a newly discovered mechanism of cell death, designated pyroptosis, also leads to specific caspase-1 activation.[47] Pyroptosis has been accepted as a death mechanism, and macrophages undergoing pyroptosis have been shown to present features of both apoptosis and necrosis with a particular activation of caspase-1[48]. Caspase-1, which was first described as the IL-1β-converting enzyme (ICE), is essential for the release of active IL-1β. In fact, caspase-1's "natural" substrates are certain members of the IL-1 family, which include pro-IL-1β, pro-IL-18, and pro-IL-33.[49,50] Cells dying by pyroptosis increase in size and show signs of plasma-membrane pores leading to increased osmotic pressure, water influx, cell swelling, and eventually osmotic lysis and specific abundant secretion of IL-1β and IL-18.[51] These features have also been shown in macrophages exposed to LtxA by our research group.[20,42] IL-1β secretion is induced robustly by extracellular ATP, which signals via the purinergic receptor, $P2X_7R$. This causes K^+ efflux from cells which activates pro-caspase-1 and consequently pro-IL-1β processing.[52] The efflux of potassium ions causes an influx of calcium ions, which in turn activate phospholipases.[53] Our research group was the first to show that the LtxA-induced pro-inflammatory cell death of macrophages involved ATP and $P2X_7R$[54] (**Fig. 2B**).

Several bacterial products, such as lipopolysaccharide (LPS), are known to increase expression of pro-IL-1β in macrophages.[55] In addition, the presence of *A. actinomycetemcomitans* has been shown to increase the expression of inflammasome components in co-cultures with macrophages.[56] However, a secondary stimulus is needed to induce a substantial secretion of the bioactive IL-1β. It has been shown that this activation can be induced by various bacterial species through activation of the inflammasome (intracellular signal-induced multiprotein complex), caspase-1 activation, and IL-1β secretion.[57] This secondary activation can be induced by direct interactions of bacteria or bacterial components with the inflammasome, such as proteins secreted by *Salmonella typhimurium*[57] or *Francisella tularensis* invasion.[58] The other system for stimulating this secondary activation has been described through cell surface interactions such as with *Listeria monocytogenes* and *Staphylococcus aureus.*[57] Several studies have been able to show that LtxA can also cause this secondary activation in macrophages that consequently leads to secretion of bioactive IL-1β.[35,42,43,54]

Macrophages play a significant role in periodontal tissue remodeling, and their ability to secrete large amounts of IL-1 indicates their involvement in the enhanced bone loss seen in periodontitis.[59,60] Moreover, IL-1 levels in gingival crevicular fluid from subjects with periodontitis are generally decreased after treatment. The levels of these cytokines in gingival crevicular fluid samples seem to play important roles for the bone resorption activity seen in periodontally diseased individuals.[43,61]

IL-1 polymorphism has been suggested to be of importance for the severity of periodontitis,[62] which could enhance the proinflammatory response from macrophages exposed to LtxA.

It is tempting to speculate that in AgP cases infected with *A. actinomycetemcomitans*, where rapid tissue destruction is observed, the LtxA-induced interaction with the accumulated macrophages in the periodontal tissues plays a crucial role in the pathogenic potential of the *A. actinomycetemcomitans* infection.

A. actinomycetemcomitans in the Pre-Clinical Phase of Aggressive Periodontitis

The association between the presence of *A. actinomycetemcomitans* in subgingival plaque and the initiation and progression of AgP has been supported by observations in several population-based longitudinal studies.[17,21,23,63,64] Disease development in terms of progression of attachment loss has been evaluated prospectively among adolescents[17,21,23] in relation to the carrier status of *A. actinomycetemcomitans*. In a recently followed West African cohort there was a significantly increased risk (OR=5.126, 95% CI [2.994 - 8.779] P <0.001) for progression of attachment loss among the *A. actinomycetemcomitans*-positive young individuals as compared with the *A. actinomycetemcomitans*-negative reference individuals.[33] The carrier status of *A. actinomycetemcomitans* in this study is based on both cultivation of this bacterium and detection method for its DNA by polymerase chain reaction (PCR) analyzes. In both methods, pooled paper point samples from the gingival crevice of the examined individuals were analyzed. All serotypes of *A. actinomycetemcomitans* were related to the progression of attachment loss in this study, but the most pronounced risk (OR) for progression of attachment loss was associated with the presence of the serotype b (OR = 7.685; 95% CI [2.835 - 20.830] P <0.001).[23,33]

The two exotoxins expressed by *A. actinomyctemcomitans*, the LtxA and the Cdt, have gained more attention since the pathogenic potential of this bacterium has been addressed.[18] The diversity of the LtxA expression by *A. actinomycetemcomitans* indicates a possible role of this toxin in the pathogenesis of periodontal disease, especially among young people. The importance of the leukotoxicity of *A. actinomycetemcomitans* and its contribution to the pathogenesis have recently been shown in a study where young individuals (mean age 15.0 years) harbouring highly leukotoxic *A. actinomycetemcomitans* (of the JP2 and of the non-JP2 genotypes) had a significantly increased risk for progression of localized periodontal attachment loss during a 2 y follow-up period.[23] A strong correlation between highly leukotoxic strains of *A. actinomycetemcomitans* and periodontitis has also been reported in Brazilian populations.[65] Different ethnic populations seem to show highly variable frequency of the JP2 clonal type of *A. actinomycetemcomitans*.[66,67] This specific genotype of *A. actinomycetemcomitans* significantly correlates to periodontal disease onset in infected individuals of African origin.[17,23,68] Subjects harboring highly leukotoxic *A. actinomycetemcomitans* are reported to be 18 times more likely to convert from a healthy status to localized aggressive periodontitis than those colonized by

strains harboring the full-length leukotoxin promoter region.[17] However, there is also convincing evidence that phylogenetically diverse strains of *A. actinomycetemcomitans* of the non-JP2 genotype can carry pathogenic potential. Non-JP2 genotypes of *A. actinomycetemcomitans* of serotype b are strongly associated with disease onset and progression in African populations,[17,23,69] but also in other populations of non-African descent worldwide.[21,68,70]

The fact that the low LtxA-producing strains of *A. actinomycetemcomitans* are present in patients with aggressive periodontal disease, as well as in healthy subjects,[18] suggests that genetic and environmental factors may interfere with the LtxA expression in individuals with various degrees of susceptibility to periodontal disease.

A. actinomycetemcomitans in the Complex Biofilm of the Periodontal Crevice

As discussed previously, the presence of *A. actinomycetemcomitans* in the oral cavity of young individuals is a strong prognostic marker for initiation and progression of AgP. The microbial composition of the biofilm of the pathogenic periodontal crevice is very diverse and complex, and it involves the presence of many different periodontal pathogens.[11] However, recent data indicate that once periodontitis has developed, the character of the periodontal infection may change over time from an infection primarily associated with *A. actinomycetemcomitans* into a more anaerobic, polymicrobial infection.[71] The subgingival environment provides a stable tooth surface and an unstable epithelial surface for the microbial colonization, the latter of which continuously desquamates.[7] Both surfaces are bathed with a continuous efflux of gingival crevicular fluid, derived from blood plasma and thus nutritionally rich in nitrogenous compounds such as amino acids, peptides and proteins. As the gingival crevice deepens, the subgingival microbial milieu becomes more anaerobic and under this condition, asaccharolytic and anaerobic and/or proteolytic bacteria such as *Fusobacterium, Eubacterium, Campylobacter, Prevotella and Porphyromonas* are found. It has previously been shown that the activity of LtxA is efficiently inhibited in the presence of black-pigmented periodontal pathogens, such as *Porphyromonas gingivalis, Prevotella intermedia and Prevotella nigrescens*.[72] In addition, *P. gingivalis* displays a competitive advantage over *A. actinomycetemcomitans* in co-cultured biofilms by inhibiting the growth and attachment of the *A. actinomycetemcomitans* cells.[73,74] These findings are supported by the negative association in subgingival samples between the presence of *A. actinomycetemcomitans* and *P. gingivalis*.[75] This indicates an important pre-clinical role of *A. actinomycetemcomitans* in AgP. However, it seems reasonable to assume that the role of this bacterium is limited in competition with the obligate anaerobic periodontal pathogens in the ecological niche of a periodontal crevice. These interactions may help to explain why several cross-sectional studies have also shown an association to other periodontal pathogens than *A. actinomycetemcomitans* in the diseased lesions of AgP individuals.[11,76]

Moreover, an inflammatory host response has the capacity to modify leukotoxicity in the pathogenic periodontal crevice. McArthur and coworkers[77] reported that lipoproteins of human serum enhanced the activity of LtxA. In an attempt to repeat this finding it was shown that exclusion of the lipoproteins in human serum did not change its LtxA activating properties of the serum.[78] The serum protease inhibitors were shown to be the responsible components for the enhanced leukotoxicity by inhibiting LtxA degradation caused by PMN degranulation.[79,80] It has also been shown that systemic antibodies against LtxA efficiently neutralize the activity of the toxin, which might interfere with the pathogenicity of *A. actinomycetemcomitans*, both locally and systemically.[81] As described in detail above, LtxA induces a massive secretion of bioactive IL-1β from macrophages. Interestingly, a receptor protein for this cytokine with regulatory properties has been identified on *A. actinomyctemcomitans*, which indicates further interactions with the host inflammatory response.[82]

Conclusion

Several different studies on various adolescent populations have shown that the presence of *A. actinomycetemcomitans* in the subgingival plaque significantly increases the risk for the colonized individuals for the initiation and progression of attachment loss. This correlation is further enhanced in the individuals that are carrying sub-types of the bacterium with an enhanced leukotoxicity. LtxA has been shown to be a virulence factor with capacity to cause imbalance in the host inflammatory response by activating and killing the immune cells in a variety of ways. The association between *A. actinomycetemcomitans* and leukotoxicity with aggressive forms of periodontitis is strong, while a possible causality is difficult to prove. The primate specificity of LtxA limits the ability to examine *A. actinomycetemcomitans* infections in animal models. In addition, the strong influence from hereditary and environmental factors in the pathogenesis of periodontitis makes causal conclusions difficult to draw. However, certain genetic host peculiarities, such as the IL-1β polymorphism, might act synergistically with the LtxA-induced pro-inflammatory response. Examination of this genetic host factor of the *A. actinomycetemcomitans*-infected individuals might help us in the future to take one step further in determination of causality and identification of risk individuals.

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