

# Endotoxin-Secreting Bacteria in Periodontal Disease

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Contributor: Maria Angeles Rojo, Manuel Garrosa, Rosalia Marcano Navascues, Damian Cordoba-Diaz

The LPS is recognized by the complex TLR4/MD2, mediated by CD14 and accessory protein LBP, which induces the activation of several transcriptional regulators like factor nuclear  $\kappa B$  (NF- $\kappa B$ ), activator protein 1 (AP-1) and interferon (IFN) regulatory factors, leading to the expression of genes involved in the host immune response.

Keywords: endotoxins ; LPS ; lipopolysaccharide ; periodontal disease ; fluoride ; therapeutic approach

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## 1. Introduction

The oral cavity is one of the areas of living organisms where the highest rates of microorganisms are located. Among them, bacteria are the most common <sup>[1]</sup>, and Gram-negative bacteria play a key role in oral infections. The virulence factors used by some of the bacteria involved in the evolution of oral infections include the release of lipopolysaccharide, a structural component of the bacterial cell wall that interacts with cells of host oral cavity connective tissue, modulating its immune response and able to cause diseases <sup>[2]</sup>.

One of the oral infections that arouses greater interest due to its epidemiology is periodontal disease and its forms, periodontitis and gingivitis. Periodontitis is characterized by inflammation and destruction of connective and periradicular tissues, resulting from the interaction between microbial factors and the host immune response, which can lead to tooth loss <sup>[3]</sup>. Literature supports the link between periodontitis and systemic diseases <sup>[4]</sup>, due to a continuous inflammation, bacterial circulation and bacterial products<sup>[5]</sup>.

Among the periodontal pathogens, *P. gingivalis* is one of the most studied, capable of releasing large amounts of external vesicles containing endotoxins <sup>[6]</sup>. It is a Gram-negative bacterium, present in patients with periodontal disease (PD) that belongs to the group of black-pigmented Bacteroides and is often presented in the form of a coccobacterium. It can produce collagenase, proteases, hemolysins, endotoxins, fatty acids, ammonia, hydrogen sulfide and indole, among other products <sup>[7]</sup>.

## 2. Endotoxin as a Component of Gram-Negative Bacteria

Lipid A is a phosphorylated glucosamine disaccharide acylated with hydroxyl saturated fatty acids <sup>[8][9]</sup>, responsible for the toxic effects of Gram-negative bacterial infections. Saturated fatty acids further 3-O-acylate the 3-hydroxyl groups of the fatty acids of lipid A <sup>[10][11]</sup>. The core oligosaccharide bonds directly to lipid A and contributes to the bacterial viability and stability of the outer membrane. This phosphorylated heterooligosaccharide is also well-preserved in the proximal area to the lipid A.

Even though the biosynthetic pathway and LPS export mechanisms are common to most Gram-negative bacteria, the detailed structure of LPS varies from one bacterium to another and this could affect the virulence. Moreover, some pathogens can modify the basic structure of their LPS during the infection <sup>[12][13]</sup>. The difference between the LPS of several Gram-negative bacteria is in the length of the fatty acid chains and these seem to be related to the pathogenicity of the bacteria <sup>[14][15]</sup>. These variations are the basis of altered host immune response <sup>[16]</sup>.

In general, endotoxins are released by secretion, in vesicles formed on the bacterial outer membrane during the bacterium growth phase or are released during cell death, damaging periodontal tissues and triggering inflammation <sup>[17]</sup>. The vesicles can deliver virulence factors and modulate the host immune system during bacterial pathogenesis. LPS also are released when the cell is chemically treated to remove this glycolipid.

The LPS of *P. gingivalis* provides integrity to the bacterium and offers a mechanism for its interaction with other surfaces, allowing for the formation of biofilms <sup>[18]</sup>. During its growing phase, pathogenicity factors are released from the outer membrane vesicles (spherical microstructural bodies) <sup>[19][20]</sup>, which are powerful stimulators of the innate immune signal transduction pathways in a tissue/cell-specific manner <sup>[21]</sup>. The *P. gingivalis* -LPS basic chemical composition is typical of

a bacterial endotoxin with a main difference: the Lipid A structure can undergo isomeric acylation in two ways, tetraacylation and pentaacylation, depending on environmental factors such as hemin levels, phosphate availability and incubation temperatures; thus, eliciting differential immunoinflammatory responses [22].

### **3. Therapeutic Approaches of Oral Diseases and Their Effect on LPS**

#### **3.1. Fluoride as Bacterial LPS Inactivator**

In general, preventive and therapeutic approach of periodontal and other oral diseases, includes plaque control to reduce microorganism aggregation. This is achieved by sustained and frequent oral hygiene employing mechanical and chemical tools to lower the viral burden. Fluoride has been widely used in dentistry due to its anticaries effect, antimicrobial properties, and desensitizing potential [23][24][25][26][27][28][29].

Madléna et al. (2012) [30] observed a decrease in plaque index, gingival index and bleeding when testing in orthodontic patients using amine fluoride and stannous fluoride (Am/SnF<sub>2</sub>) toothpaste in combination or not with a mouth rinse with Am/SnF<sub>2</sub>. Under these conditions, the dental plaque shifts towards less acidogenic, thus supporting an antibacterial property of fluoride agents [31][32].

Haught et al. (2016) [33] applied antimicrobial solutions, containing stannous fluoride (SnF<sub>2</sub>), to LPS from *E. coli* and *P. gingivalis* to determine, by fluorescence assays and mass spectroscopy, the binding ability of SnF<sub>2</sub>. Stannous fluoride interfered with LPS and inhibited the binding to TLR4 in both dying and cellular assays, hence, potentially reducing their effect in the host cells. In another study, stannous fluoride inhibited gene expression response of TLR4 and TLR2 in HEK293 cells, producing a complete inhibition at micromolar concentrations. Moreover, the addition of stannous fluoride suppressed production of TNF $\alpha$ , IFN-g, IL-12p70, IL10, IL-1b, IL-2 and IL-6, and increased secretion of IL-8. Thus, stannous fluoride had the potential to provide benefits in the early signs of periodontal disease, directly decreasing the pathogenicity of plaque biofilms by blocking reactivity of LPS with tissue receptors associated with inflammation [34].

Clinical improvements in gingivitis also have been reported after applying stannous fluoride dentifrice [35]. These authors found significant changes in the number of cultivable Gram-negative organisms in sampled supragingival and subgingival plaque and a considerable reduction in promotion of TLR activation for subgingival plaque samples. Furthermore, Xie et al. (2018) [36], in an expanded analysis of previous studies, observed the hygienic treatment effects of stabilized stannous fluoride tooth paste on chemically measured endotoxins and the activation of TLR based gene expression in TLR2 specific cell line and a THP-1 (multi TLR reporter) cell line. These authors found that SnF<sub>2</sub> dentifrice treatment potentially reduced the endotoxin content and virulence potentiation properties of subgingival dental plaque, therefore concluding that SnF<sub>2</sub> might be beneficial to reduce the pathogenicity of subgingival dental plaque.

#### **3.2. Surgical and Non-Surgical Periodontal Treatment as a LPS Modulator**

Authors agree that periodontal treatment can reduce the bacterial burden and therefore the level of LPS, attenuating their potential inflammatory effect in short term. The therapeutic approach, consisting in professional plaque removal together with home-care reinforcement, combined with an antibiotic treatment based in 500 mg amoxicillin and 250 mg metronidazole, three times a day for seven days, was effective in partially modulating LPS responsiveness [37].

Following the periodontal treatment protocol for patients with localized aggressive periodontitis (LAP) described by Shaddox et al. (2013) [38], in which an ultrasonic full-mouth debridement, site-specific scaling and root planning was performed together with the prescription of wide spectrum antibiotics, combined with home-care instructions, a decrease in clinical parameters of the disease was observed probably due to a reduction in the cytokine/chemokine LPS response after treatment [39]. These studies refer to a rebound effect in LPS inflammatory response after six to twelve months, that apparently do not influence the ability of high responsive patients to show reductions in their clinical parameters of disease, likely due to LPS tolerance in these subjects [40][41][42].

#### **3.3. LPS Neutralization by High Density Lipoproteins**

Most of lipoproteins have been reported to bind LPS. The high-density lipoproteins (HDLs) seem to be the more efficient for binding and inactivating different types of LPS. According to Meilhac (2012) [43], the plasma phospholipid-transfer protein (PLTP) was then reported to transfer LPS to HDL in conjunction with the LPS-binding protein (LBP) leading to LPS neutralization. LBP and PLTP can remove LPS from bacterial membranes and transfer it to HDLs [44][45]. Lipid A diglucosamine-phosphate region seems to be responsible for the association of LPS with HDLs and its neutralization

relies on LBP, which may form a complex between CD14 and LPS, favoring its binding to HDL particles and subsequent neutralization, revealing a protective and possible contributory potential in periodontal disease treatment <sup>[46][47]</sup>.

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